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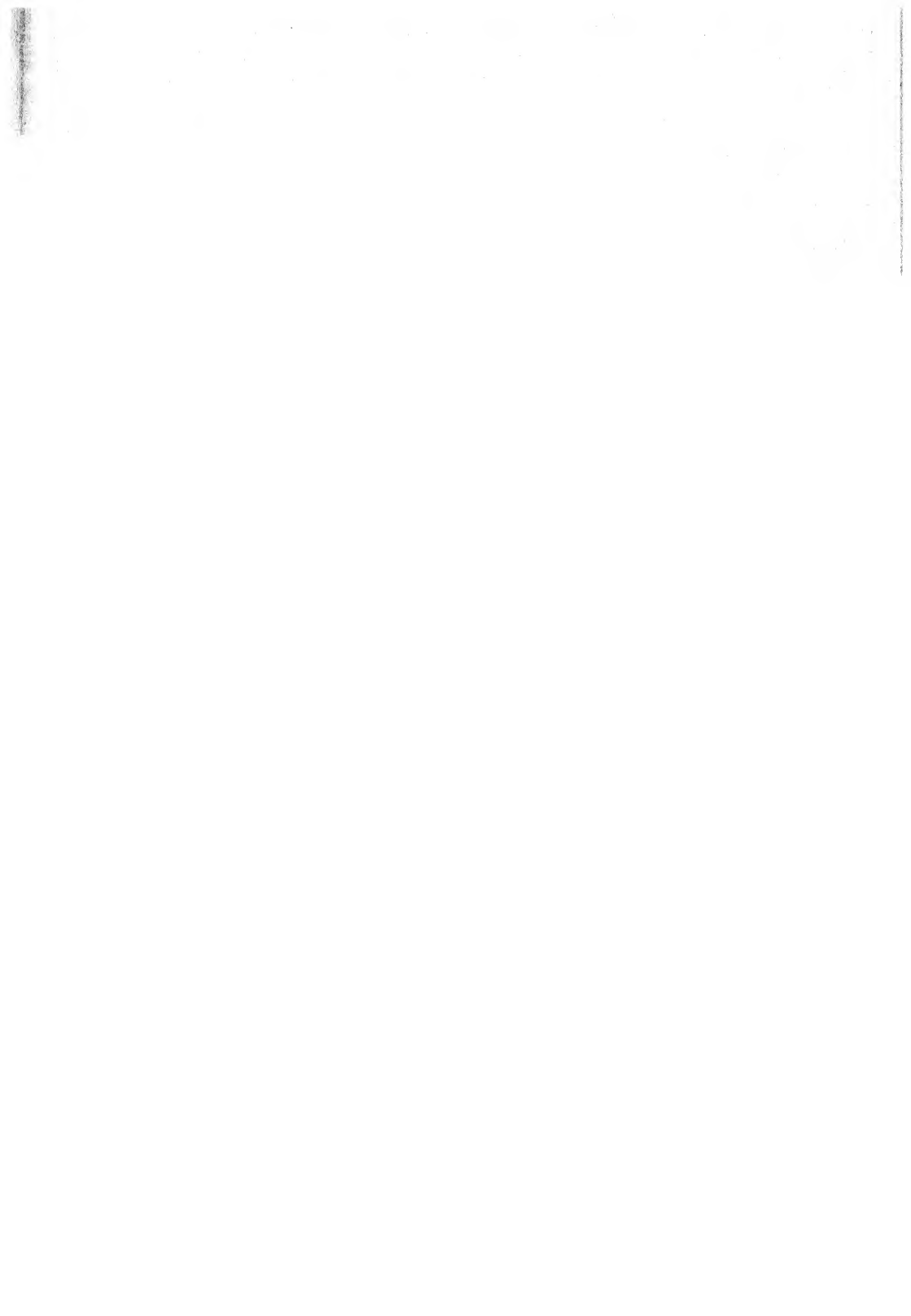
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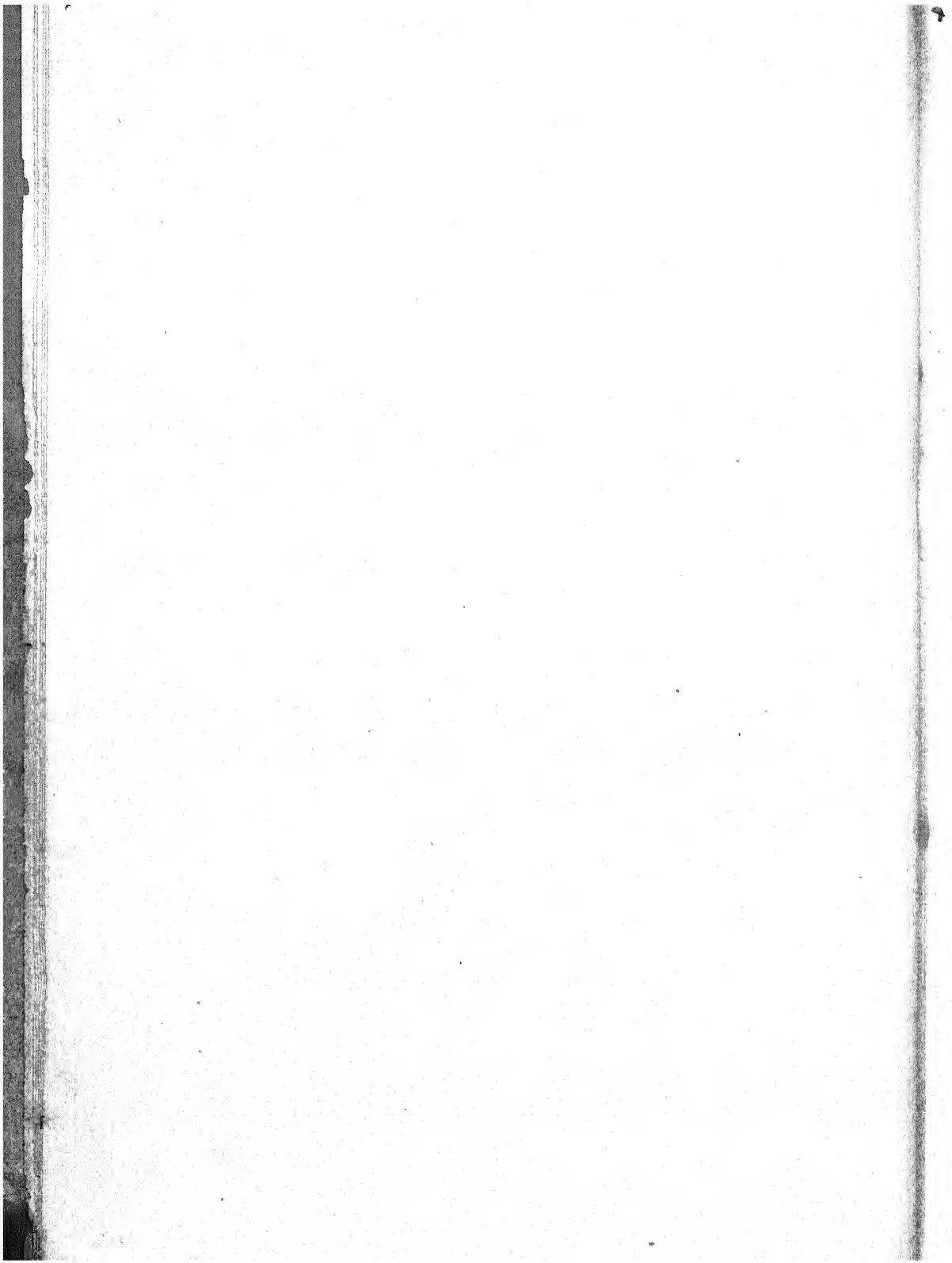
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NUMBER 1

THE INFLUENCE OF BIOS ON NODULE BACTERIA AND LEGUMES¹

A. THE INFLUENCE OF BIOS ON LEGUME SEEDLINGS

BY D. G. LAIRD² AND P. M. WEST³

Abstract

The hypocotyls of red clover seedlings, when sprouted on a seed bed enriched with crude Bios 2, grew upwards in a vertical position, while the cotyledons rested on the surface, supporting the inverted plant and possibly absorbing nutrients for its growth. From the tip of the upturned primary root, secondary roots developed about ten days after seeding. These new roots grew downwards, and after approximately one week, they penetrated the substratum in a normal fashion.

The concentration of crude Bios 2 necessary to cause maximum hypocotyl bending, was observed to be approximately four times that required to produce optimum stimulation of the nodule bacteria.

When plants were allowed to start in an unenriched growing medium superimposed on a layer of agar enriched with crude Bios 2, no upward bending of the roots occurred as they reached the Bios layer.

Bios 2(b) alone appears to be the factor responsible for the hypocotyl bending phenomenon. Bios 1, Bios 2(a), pantothenic acid, various amino acids, and miscellaneous compounds were tested and did not appear active in this respect.

Though causing a different form of root aversion, hetero-auxin, like Bios 2(b), actually prevents the hypocotyls from entering an enriched medium. While the two substances bring about a certain similarity of physiological effect, they cannot be considered identical, and chemically, they are absolutely distinct.

A drop of Bios 2(b) placed on the sensitive parenchymous lining of a bean pod caused rapid cell multiplication, resulting in the production of a wart-like protuberance. Similar, though somewhat less distinct results followed the pricking of such tissue with a pin. These results would appear to strengthen the hypothesis advanced by Went in which Bios was assigned the properties of a "wound hormone".

A study devoted to the influence of the Bios complex on nodule bacteria, alone and in association with red clover (unpublished data), suggested to the writers that Bios might exert a specific influence on the character of the host. While the Bios of Wildiers is commonly known to occur in the tissues of higher plants (4, 5, 11), its effects on the growth processes of such plants are as yet unknown. Kogl (6), has suggested that Bios may be physiologically related to auxins (a) and (b), and hetero-auxin, which appear to function both as organizers and as phyto-hormones of cell expansion. Went (12), on the other hand, has presented the hypothesis that Bios might be considered

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a wound hormone. A consideration of these facts and suggestions, and a desire for increased knowledge regarding the important interrelationships between bacteria and legumes have been the inducements for a study, as reported below, of the influence of Bios on the growth of red clover seedlings.

The Bios fractions used in preliminary work by Eagles *et al.* (3), were prepared after the manner of Miller and his associates (9). As the work progressed, however, the original procedure for the isolation of Bios concentrates was altered in order to obtain fractions with fewer impurities. These efforts resulted in the development, by Eagles and Wood (unpublished data), of an improved procedure. The Bios 2(a) and 2(b) concentrates prepared according to the new method were found to exercise the same biological effects as the respective fractions prepared after the manner of Miller when tested on bacteria, but possessed a distinctly greater activating capacity. Bios obtained by the revised procedure of fractionation was used in the plant studies subsequently reported upon.

The following method was employed to determine the influence of Bios 2 preparations on the growth of red clover: Using washed sand cultures, the seeding was carried out under aseptic conditions; the required nutrients were supplied in the form of modified Crone's solution (2), containing, except in the case of the controls, 2% of an active solution of Bios 2, *i.e.*, a solution containing both Bios 2(a) and Bios 2(b). The rate of germination was normal, but the seedling roots growing on a Bios medium absolutely refused to penetrate the sand, with the result that they were unable to support the plant. After the surface of the sand had dried slightly, the plants wilted and died. This experiment was conducted in 4-inch porcelain pots, each treatment replicated four times, and about 50 seedlings grown in each pot. The test was repeated to guard against any possible error.

As sand did not appear to constitute a satisfactory growing medium, it was replaced by a modified Crone's nutrient solution to which was added 0.75% Bacto agar. The Bios enrichment used was the same as in the previous experiment. Red clover seeds were then placed on the surface of the medium which had been autoclaved in quart sealers covered by a Petri plate. The effect of the Bios became evident after three to four days. In every case the hypocotyls ascended vertically or nearly so, while the cotyledons remained in contact with the agar surface (see Fig. 1). This phenomenon is referred to throughout the remainder of the paper as "root bending", since it is the root tissue of the hypocotyl that undergoes subsequent modification and not the stem tissue. The upturned hypocotyls differed further from the normal in being slightly swollen and in possessing no root hairs. The upward growth continued for approximately ten days, and after that, one or more secondary roots were produced which grew downwards and penetrated the substratum in an apparently normal fashion. In most cases, the first true leaf and the subsequent trifoliate leaf had developed when the secondary roots entered the nutrient medium (see Fig. 2). Until this time, the absorption of water and any possible nutrients required must have taken place through the coty-

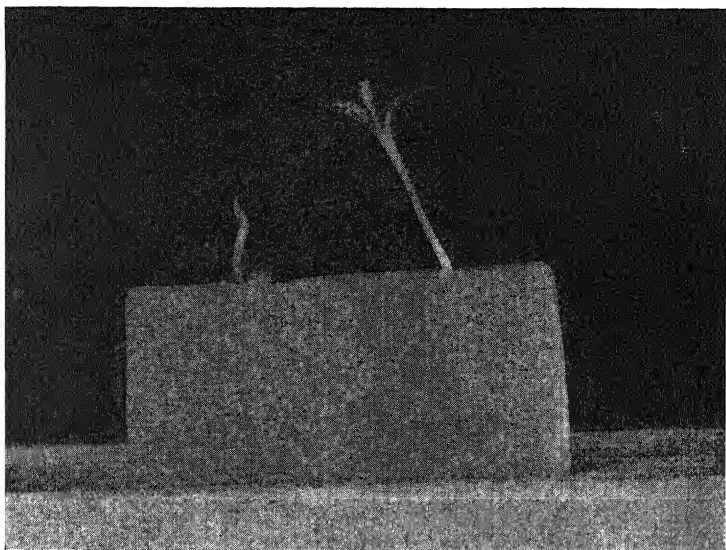


FIG. 1. Red clover seedlings 7 days old. Left: typical upward growth of hypocotyl on medium enriched with Bios 2. Right: normal growth on unenriched control medium.

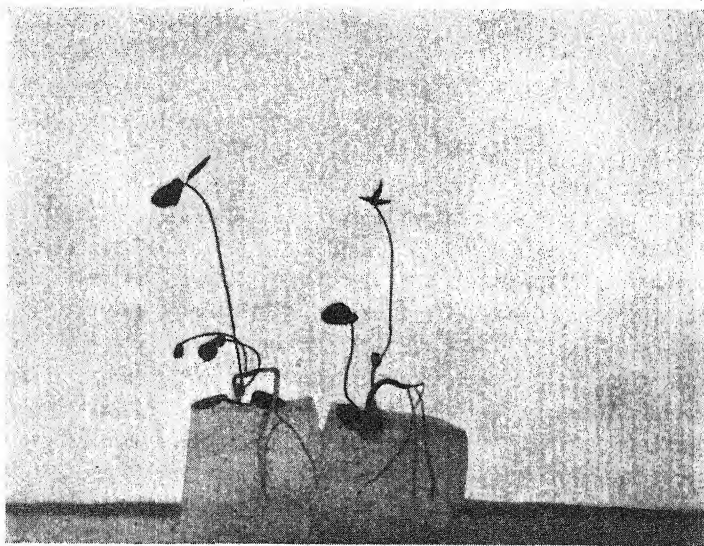


FIG. 2. Red clover seedlings 21 days old, on Bios 2 enriched medium. Note secondary roots growing down in a normal fashion through the medium which caused hypocotyls to bend upwards.

ledons, or the stem immediately beneath them, or through both. Thousands of seedlings have been used in this study. The experiment has been repeated numerous times and always with identically the same result. In fact, in work now in progress, the hypocotyl behavior as described is being used in determining the character of certain fractions as isolated from plant and animal materials.

It became evident, during the investigation, that red clover seedlings vary in their response to Bios enrichment, and this observation led to a detailed study of the effect of different Bios concentrations. The concentration of any preparation required for maximum "root bending" was observed to be roughly four times that required to produce an optimum stimulation of the nodule organism. Fractions of unknown activity were, therefore, first tested on bacteria to obtain an indication regarding the amount required to produce "root bending". By varying the concentration of Bios in the medium from 0.25 to 8% the maximum "root bending" effect was observed to occur with a 2% enrichment. Beyond 2% there was evidence of slight toxicity, while below 2% only the more "sensitive" plants responded, the roots of the others merely extending along the surface of the substratum. Since the activity of different Bios solutions may, and do, vary to some extent, the above figures refer only to the preparation used in these experiments. While a positive correlation was observed to exist between the Bios concentrations required for optimum bacterial growth and "root bending", the possibility is not to be overlooked that more than one factor might be concerned.

Likewise, it seemed desirable to determine the effect of Bios on the growth of plant roots after the plants had become established in the absence of Bios. For this purpose, a layer of Crone's agar medium containing Bios 2 enrichment in optimum concentration was placed in the bottom of a quart jar, and another layer, without Bios addition, was superimposed on this. Seeding was carried out as previously described. The seedlings grew normally, and when, after one week, the roots reached the layer containing Bios, they neither turned up nor did they show any other observable effect from the Bios concentration. It would seem probable, under certain conditions at least, that the factor which causes such marked changes in the hypocotyl as observed in the primary stages of growth, does not exercise any influence on the root tissue once the early seedling stage has passed.

Although, when tested separately on bacteria, no distinct difference had been observed in the action of Bios 2(a) and Bios 2(b), it was considered possible that one or the other of these factors might be specifically responsible for the "root bending" phenomenon. Experimental results showed that an agar seed bed enriched with Bios 2(b) alone brought about bending of the hypocotyls, while the addition of Bios 2(a) alone exercised only a very slight influence, if any, in this respect. Crude Bios 2(a) and 2(b) preparations obtained after the manner of Miller *et al.* (9), were likewise tested and gave similar results, though crude Bios 2(b) was not as active in producing "root bending" as the more refined Bios 2(b). This finding lends support to the

conception of Miller that Bios 2 is of a multiple nature, and it further indicates that the new fractionation method results in a reasonably complete separation of two distinct entities as represented by Bios 2(a) and 2(b) respectively.

Recently Miller (10), made the suggestion that the active constituents of Bios 2(a) might be β -alanine and *l*-leucine. These amino acids were included in the tests along with aspartic and glutamic acids, arginine, cystine, tyrosine, tryptophane, and histidine; none of these compounds caused bending of the hypocotyls. Negative results were also obtained with Bios 1 (inosite). Similarly, the pantothenic acid of Williams which has been reported to stimulate the growth of alfalfa (8), and Riccia (13), did not possess the effect of Bios 2(b) as shown on red clover plants. Various compounds, carnosine, indole, scatole, guanine, ergothioneine, and glutathione were also found to be inactive.

In order to determine whether or not the "root bending" phenomenon might be due to the toxicity of the heavy metals used, and possibly carried through in small quantities into the Bios preparation, these metals (barium, mercury, and lead) were added to the medium in minute amounts. General evidence of toxicity was observed, but otherwise the metals caused no change in the normal root development.

In the light of the work of Kogl (6, 7), and Went (12) regarding the effect of auxins on plant growth in general, it was thought desirable to enquire whether or not a corresponding "root bending" phenomenon might be caused by these substances. *A priori* it was realized that the treatment of auxins with heat, in the presence of dilute acid and alkali respectively, results in their destruction, while Bios 2(b) is quite stable under similar conditions. Besides, auxins are soluble in ether, and Bios 2(b) is not. Thus on the basis of chemical properties it did not seem probable that any of the known auxins could be responsible for the "root bending" of the type referred to above. However, the response of red clover seedlings to hetero-auxin (β -indolyl acetic acid) was studied in a manner similar to that already described for Bios, except that while the latter was used in a 2% concentration, the hetero-auxin, after being tested at six different concentrations ranging from 0.0001 to 0.01 mg. per cc., was used at a concentration of 0.0005 mg. per cc. Germination of the seeds revealed, that while hetero-auxin, like Bios 2(b), does not permit the hypocotyls to enter the substratum, there are, at the same time, apparent differences in the action of the two agents on red clover seedlings. As previously reported by other workers (12), hetero-auxin causes the development of thick, heavily swollen hypocotyls not observed in the case of Bios 2(b). The latter, again, compels the hypocotyls to extend upwards from the cotyledons, which remain resting on the seed bed, while hetero-auxin, although not permitting the hypocotyls to enter the seed bed, causes a slight horizontal twisting of the hypocotyls and allows the cotyledons to occupy their normal upright position. Finally, Bios 2(b) inhibits the formation of root hairs on the primary roots, while hetero-auxin has no such inhibitory influence. Hetero-auxin and Bios 2(b) may, therefore, be distinguished on the basis of either their chemical properties or their physiological effects on the development of red clover seedlings.

After having demonstrated the dissimilarities between the auxins and Bios 2(b), the attention of the writers was called to the recent work of Bonner (1) who, in seeking an ideal medium for plant tissue cultures, found that additions of plant extract were essential to the successful growth of his cultures. His statement regarding the properties of the active compound in the extracts employed indicates a close relationship to Bios 2 fraction, and his reference to the adsorption of the active substances on charcoal would suggest that the compound with which he was concerned might be identical with Bios 2(b). By applying a drop of his extract to the parenchymous lining of the cup-like depressions inside a bean pod, Bonner reports the production of a "wart-like protuberance". A similar effect was observed following the pricking of such cells with a pin. The auxins, vitamins B₁ and B₂ and pantothenic acid were shown by him to be inactive in this respect.

On the assumption that Bios 2(b) might be the causative factor, the writers conducted a similar test using this activator, with hetero-auxin, water, and pin-pricks as controls. Bios 2(b) produced protuberances which appeared to be similar in all respects to those described by Bonner, while hetero-auxin and water were ineffective. Histological preparations showed that an intensive cell multiplication occurred from the addition of Bios 2(b) where *Phaseolus vulgaris* was tested, while in the case of *Phaseolus coccineus*, cell elongation was chiefly responsible for the swelling. Pin-prick controls gave evidence of a small amount of cell multiplication. These results would appear to strengthen the hypothesis advanced by Went in which Bios was assigned the properties of a "wound hormone".

Acknowledgments

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ACTIVITY OF THE VASCULAR CAMBIUM IN RELATION TO WOUNDING IN THE BALSAM POPLAR, *POPULUS BALSAMIFERA* L.¹

BY A. B. BROWN²

Abstract

An investigation of the response of the vascular cambium to wounding in leader shoots of the balsam poplar, *Populus balsamifera* L., was carried out in the laboratory during the winter months, at which time observations on local wound cambial activity, distinct and apart from normal cambial activity, can be obtained. It was found, in disbudded units, that the greater the amount of living bark distal to a wound, the greater is the development of local cambial activity in relation to the wound. Local wound cambial activity is further promoted by the presence of developing buds and leaves distal to the wound, and the effect can be observed before the basipetal gradient of normal cambial activity emanating from the developing extension growth has reached the wound. Cambial activity in relation to wounding responds to gravity in the same way as normal cambial activity. In horizontally placed leader shoots, cambial activity is greater in relation to a wound on the upper side of the shoot than to a similar wound directly opposite on the lower side. On the basis of these results, it is suggested that a hormone, present in the living bark and also produced by developing buds and leaves, is involved in local wound cambial activity. In all probability this hormone is identical with that which promotes normal cambial activity.

It is also suggested that a wound substance, capable of promoting by itself cell division only, is involved in local wound cambial activity. The amount of this wound substance produced is apparently proportional to the extent of dying of the cells of the bark subsequent to wounding. From the lower edge of a complete ring, a very feeble basipetal gradient of cambial activity arises, in which differentiation to form vessels and fibres does not occur, although a few tracheids may be found. This type of behavior has not hitherto been reported, and is interpreted as the result of stimulation of the cambial layers by the wound substance alone. Local cambial activity above a complete ring and in relation to bridged wounds, involves differentiation of more or less typical vessels and fibres, and is interpreted as the result of interaction between the wound substance and the cambial hormone traveling basipetally in the living cells of the bark. The absence or feeble development of cambial activity at certain points in relation to bridged wounds, in contrast to greater development at other points where presumably the concentration of cambial hormone must be less, is interpreted as the result of lack of wound substance or low concentration of it acting as a limiting factor.

Introduction

For a long time it was thought, and apparently still is by many, that the vascular cambium plays an important role in the formation of callus tissue, but this belief derives no support at all from the recent work of Sharples and Gunnery (19), and of Sass (18). According to these investigators, callus tissue arises as the result of proliferation of medullary ray cells particularly, and not from the vascular cambium generally. However it is known definitely that the cambium responds to wounding, under certain conditions, to produce vascular elements.

Hartig (9) was the first to observe the basifugal development of cambial activity from the upper edge of a complete ring in woody shoots, and Sledge (20) has greatly increased our knowledge of this phenomenon in recent years.

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This upward spread is of limited extent, and is quite independent of the presence of growing buds and leaves, upon which the normal basipetal development of cambial activity in the shoot does depend. The writer (2) has also observed the same type of thing in ringed roots of poplar. Swarbrick (24) mentions an interesting case of local cambial activity as a result of wounding, in a completely disbudded apple shoot. In one of his experiments a small triangular piece of cortex and phloem was accidentally torn out, so as to leave the xylem exposed. Upon examination some time later, cambial activity was observed above, below, and lateral to the wound.

A major communication on the subject of tissue reorientations in woody plants, in response to wounding, has been contributed by Janse (10). He conducted an extensive series of ringing and wounding experiments, and appears to have been the only investigator to pay any attention to the behavior obtaining *below* bridged rings and other types of wounds. The main value of his publication lies in his observations as such and the abundance of excellent photographs illustrating them, rather than in his interpretation of his results. Apparently, his experiments were carried out during the growth period when normal cambial activity was proceeding, and he has nothing to say about local wound cambial activity independent of normal cambial activity. He showed, among other things, that when an oblique bridge of bark was left connecting the upper and lower edges of a ring, the cells within the bridge soon became reoriented to run in the same direction as the bridge, and that this change was a gradual one, becoming more and more marked in each successive layer of cells cut off by the cambium. A little later Teodoresco and Popesco (25) studied callus formation and the reaction of the cambium in relation to a ring in which a bridge of bark in the form of a step (Czapek's ring) was left, connecting the upper with the lower edge. The wound was made when the cambium was active, and they found that ultimately the elements in the horizontal part of the bridge became reoriented to run transversely, with the result that uninterrupted vascular continuity between the parts above and below the wound was re-established. This type of behavior is also discussed by Priestley (15) in relation to his theory of symplastic growth, and a detailed investigation of the changes involved in the reorientation of the cambium within the bridge has been carried out by Tupper-Carey (27). Somewhat similar results, although less detailed, are reported by Collins (4), who found that he could obtain zig-zag and spiral grain of the wood as a result of appropriate wounding.

This present investigation involves a critical study of the reaction of the vascular cambium to different types of wounds made in shoots during the winter period. In this way it was possible to study local wound cambial activity for a considerable period of time before the normal basipetal flow of cambial activity from developing extension growth had reached the wound, and in completely disbudded units, apart altogether from normal cambial activity. A number of significant correlations have revealed themselves, on the basis of which the response of the vascular cambium to wounding is

interpreted in terms of the interaction between a hormone present in the living bark, which is very probably identical with that promoting normal cambial activity as described recently by Snow (23), and a wound substance produced subsequent to wounding.

Material

The material used throughout this investigation was *Populus balsamifera* L., the balsam poplar. Main stems or leaders only were employed, and some 600 trees, yielding more than 1,000 experimental units, have been involved in the work. The units practically always consisted of a length of shoot derived from the extension growth of one particular year, and varied in age from one to four years. All the experiments were done in the laboratory during the winter months, from December to May, during which time the cambium was dormant in material in the field. The wounds were smeared with vaseline and the shoot placed vertically with its basal end immersed in water, where rooting took place very readily as the result of rapid development of preformed root initials. Normal bud-break took place in the laboratory, and leaves began to appear about two weeks after the units had been set up in water. In all the experiments to follow the shoot was placed vertically, unless otherwise stated.

It was found that by peeling the bark from the shoots, at the end of the experimental period, and allowing them to dry out, the new xylem formed locally subsequent to wounding showed up clearly upon the surface of the old wood. This procedure was used extensively throughout the investigation and proved to be of great value.

Qualitative Experiments

(a) *Activity Above and Below a Complete Ring*

Cambial activity immediately above a complete ring in balsam poplar is essentially similar to that already observed by Hartig (9) and Sledge (20) in other woody plants. It is a local development, independent of normal cambial activity emanating from developing extension growth, and is expressed by a very obvious basifugal gradient of xylem formation, spreading for a short distance distally from the upper edge of the ring. There is a marked tendency for this new xylem to be "piled up" just above the ring. In appearance this wood is not markedly atypical, at all events in transverse section. It consists of vessels, tracheids and well thickened fibres, with nests of parenchyma not infrequently included. The most obvious departure from typical stem wood is to be found in the shape of many of the vessel segments. They are narrow, and might readily be identified in longitudinal sections as tracheids with long tapering ends. On maceration of the wood, however, these elements reveal themselves as vessel segments with very oblique end walls which are for the most part pitted, and the perforations between these vessel segments occur laterally at maturity, whatever their place of origin may have been morphologically.

The general belief is that no cambial activity originates from the lower edge of a complete ring, but this is certainly not the case in balsam poplar, where it was found that a very feeble basipetal gradient of cambial activity does develop from the lower edge of a complete ring. Apparently the cambium cuts off cells mostly to the inside. In transverse section (Plate I, Fig. 3) these cells are more or less uniformly rectangular in shape. They remain thin walled and unligified with the exception of occasional small groups which lignify, without appreciable expansion in any direction, to form scalariform and reticulately pitted tracheids. Vessels and fibres have not been found to occur. As many as 10 to 15 rows of cells, cut off to the inside in this manner by a well defined cambium, have been observed just below the lower edge of a complete ring. The gradient falls off rapidly, and usually within the space of a few millimetres ceases to be easily detectable.

It has, however, been observed clearly in many cases for a distance of 1 cm. from the lower edge of the ring. Relative to that obtaining just above a complete ring, the amount of cambial activity developing basipetally from the lower edge is indeed small. As far as the writer is aware this type of development has not been reported before. Sledge (20), in one case only, an internodal cutting of *Sambucus*, detected cambial activity at the distal end, where a few new elements had been cut off at one place, but apparently not completely around the shoot as in poplar.

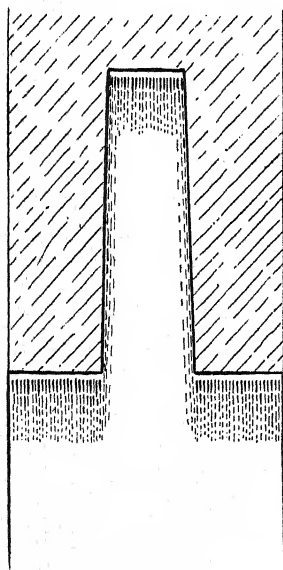


FIG. 1. Illustrating greater development of cambial activity longitudinally from the transverse margins than laterally from the longitudinal margins, in a wound where a longitudinal tongue of bark runs distally up the shoot, from what is really the lower edge of a complete ring.

A simple modification (Fig. 1) of the complete ring yielded results of marked significance. In this case the ring was very wide, and a tongue of bark (cortex and phloem) was left, running distally from the lower edge. After some weeks the material was examined for cambial activity, with the result expressed by the dotted lines in the diagram. A feeble basipetal development of cambial activity was detected spreading downwards from the transverse margins, but extremely little laterally from the longitudinal edges of the wound. The elements formed were of the type already described as occurring below an ordinary complete ring, *i.e.*, unligified, with the exception of a few tracheids. A correlation that may be of some significance in this connection is that dying subsequent to wounding is always more extensive longitudinally from the transverse edges of the wound, than it is laterally from the longitudinal edges.

No evidence was obtained that cambial activity from the lower edge of a complete ring is dependent upon the development of adventitious buds, which occasionally arise from the callus tissue in this

region. Such buds promote a local obliquely basipetal development of cambial activity in the shoot below them, but this activity is easily distinguished from the uniform development that is to be observed when no adventitious buds are present. Moreover there was no question of any stimulus to cambial activity passing across the ring, for precisely the same type of behavior can be observed at the distal end of completely disbudded cuttings. The foregoing results are also of interest when compared with callus formation in relation to ringing. Callus formation, just as cambial activity, is more marked at the upper edge of a complete ring than it is at the lower, where it does however usually occur to some extent.

(b) *The Longitudinal Bridge*

The following observations apply to local cambial activity in relation to a wound where, instead of making a complete ring, a longitudinal bridge of bark is left, connecting the parts above and below the wound. In some shoots a complete ring was made some distance above the wound under consideration, and the shoot completely disbudded and lateral branches removed between the complete ring and the wound below. In others no complete ring was made above the wound, but the shoot was disbudded and laterals removed for a considerable distance above the wound. This was done in order to make it possible to observe local wound activity for some considerable time before the normal basipetal development of cambial activity from the developing extension growth reached that point. Actually however the sequence of events was exactly the same in both groups. Fig. 2 and Plate 1, Fig. 1 illustrate the result obtained in relation to a wound of this type after two to four weeks. The arrangement depicted is visible to the naked eye when the bark is peeled off and the shoots allowed to dry out. It will be observed that just distal to the upper edge of the wound, a well defined basifugal gradient of xylem has been laid down, essentially similar to that obtaining above a complete ring. Within the bridge, however, cambial activity is basipetal, being undoubtedly more evident at the distal end of the bridge during the earlier stages of development. In transverse sections through the bridge it will be found that wedges of new wood, tapering off to the inside from the edges of the bridge, have been formed. Below the bridge, cambial activity swings round on both sides to run obliquely down and around the shoot. The first signs of cambial activity are always to be observed just distal to the upper edge of the wound. Notice (Fig. 2) that there is no cambial activity in the median line of

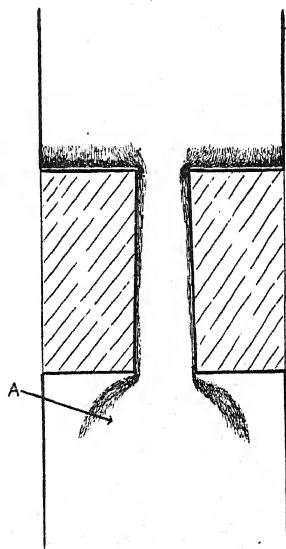


FIG. 2. Local cambial activity in relation to a longitudinally bridged ring, expressed in terms of xylem formation as revealed when the bark is peeled from the shoot.

the bridge itself, or in the shoot above and below in the same longitudinal line as the bridge. Particular attention should be paid to the fact that there is no cambial activity in the region A (which is definitely lateral to the longitudinal bridge), whereas cambial activity does occur lateral to it, not only at the same transverse level but also below that level. The obliquely basipetal spread below the wound runs for a greater distance down the shoot than the basifugal development above the wound extends up the shoot. However, the depth of wood radially is always greater just above the upper edge of the wound than at any other point. Another especially interesting feature is that there is definitely more cambial activity (including cell divisions, vessel formation and lignification) below the wound than within the bridge itself. This is very different from the state of affairs obtaining in relation to a similar wound coming under the influence of the normal basipetal development of cambial activity for at least some considerable time. The writer (2) has observed in aspen poplar, that in such a wound either in the shoot or the root, the width of the growth ring is always greater within the longitudinal bridge than it is above or below. Teodoresco and Popesco (25) also make a statement to the effect that the amount of new xylem and phloem formed within the zig-zag bridge in their experiments was abnormal, as if an attempt were being made to replace the tissues which had been cut away.

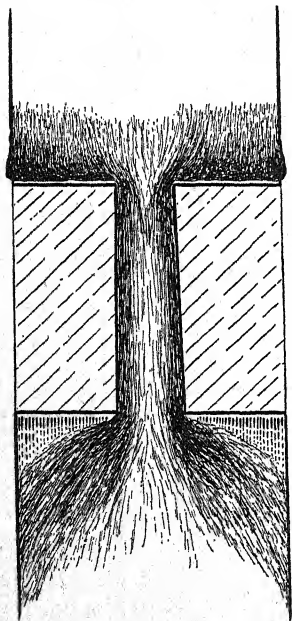


FIG. 3. Same as Fig. 2, but showing more extensive development. The dotted lines indicate feeble cambial activity similar to that occurring below a complete ring, in which there is no differentiation of vessels or fibres.

Development has been followed for as long as eight weeks, but no significant changes occur. There is a general increase in the amount of cambial activity (Fig. 3). The wedges of xylem within the bridge ultimately meet, provided the bridge is not too wide and, again depending upon the width of the bridge, the depth of wood may become more or less uniform across the bridge. Coincident with this, new xylem may make its appearance to a greater or less extent, above and below the wound, in the same longitudinal line as the bridge. The obliquely basipetal development below the wound becomes more extensive, and swings round on both sides to meet behind. At the end of eight weeks, in those shoots with no complete ring above the wound, the normal basipetal development of cambial activity from the buds and leaves had usually reached the wound, but even then, in this material, there was still less cambial activity within the bridge, relative to that obtaining below the wound.

The xylem developing basifugally from the upper margin of the wound is essentially similar to that above a complete ring, consisting of vessels

(mostly atypical and of the type already described), tracheids, fibres and included groups of parenchyma. Within the bridge the new wood is usually more or less normal, except for the not infrequent presence of parenchyma. The obliquely basipetal development below the wound is, however, particularly interesting. Actually in that sheet of tissue the cells are all oriented, at least for a very considerable period of time, in the normal longitudinal direction. Undoubtedly development is obliquely basipetal, but it would not be so obvious to the naked eye perhaps, were it not for the peculiar mode of development of the vessels. In this new wood, vessels do not arise from the progressive vacuolation of vessel segments in longitudinal series, but rather from a series of cells running obliquely down and around the shoot. The result is that it is only the vessels as a whole that run obliquely basipetally, whereas the vessel segments themselves, the fibres, the un lignified parenchyma and the medullary rays practically retain their normal orientation (Plate I, Fig. 5). The vessel segments are atypical, however, in that the end walls are definitely more oblique than usual, and the perforations between vessel segments tend to be lateral at maturity. Moreover the vessel segments are often shorter than those of the typical vessel. Even after as long as eight weeks, very little progress in the direction of a general reorientation of the tissue elements was observed in this material. A general reorientation is however complete within a much shorter period of time below a similar wound in the normally growing tree, during the season of active growth. These observations would seem to indicate that the process of vessel formation plays rather an important part in tissue orientations of the type just described.

There still remains for consideration one important point in connection with the longitudinally bridged wound. In that area below the wound which is missed by the strong obliquely basipetal spread, one can detect a very feeble basipetal development of cambial activity from the lower transverse margin of the wound. This development, which is by no means obvious to the naked eye, is represented in Fig. 3 by the dotted lines, and is exactly the same as that arising from the lower margin of a complete ring (c.f. Fig. 1), in which vessels and fibres do not differentiate (c.f. Plate I, Fig. 3). Actually this basipetal development can be detected most readily in that region of the shoot, behind and below the longitudinal bridge, where the distance between the lower transverse margin of the wound and the strong obliquely basipetal spread is greatest. Within the bridge itself, the exceedingly feeble stimulation of cambial activity laterally from the longitudinal margins, corresponding to that obtaining in a distally running tongue of bark, as previously described (Fig. 1), is completely masked by the stronger basipetal development down the bridge.

It may be stressed at this time, that a similar feeble basipetal development of cambial activity, in which vessels and fibres do not differentiate, can be detected below all bridged wounds, in that region missed by the strong obliquely basipetal spread which is characterized by differentiation of vessels and fibres. For simplicity, however, this development will not be depicted in

the drawings to follow, in which only the obvious cambial activity, expressed in terms of xylem formation, involving differentiation of vessels and fibres, will be illustrated.

Fig. 4 illustrates cambial activity in relation to another wound which is really a modification of the type just considered. In this case a rectangular piece of bark has been cut out. It is hoped that the drawing

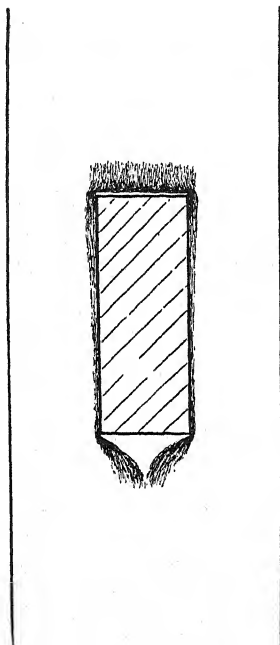


FIG. 4. Local cambial activity in relation to a rectangular wound, expressed in terms of xylem formation.

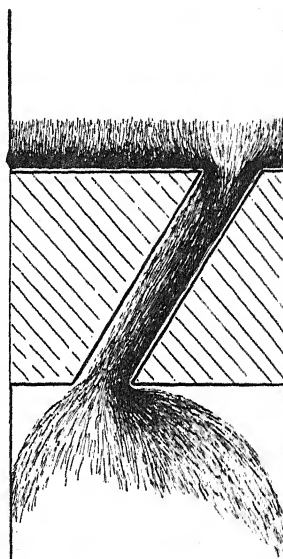


FIG. 5. Local cambial activity in relation to an obliquely bridged ring, expressed in terms of xylem formation.

more or less explains itself and nothing more will be said regarding it. An essentially similar arrangement is obtained around the wound resulting from the removal of a circular piece of bark.

(c) *The Oblique Bridge*

A series of experiments was also set up, in which an oblique or spiral bridge of bark, instead of a longitudinal one, was left connecting the upper and lower edges of the wound (Fig. 5). It is proposed to allow the diagram to tell its own story more or less, which permits the following descriptive remarks to be brief. Here again a basifugal gradient of xylem is laid down from the upper edge of the wound. Development below the wound is obliquely basipetal, just as in the previous experiments. Within the bridge itself development is also obliquely basipetal, the grain of the wood running parallel to the edges of the bridge. The following significant points should be noted. Within the bridge cambial activity is most marked at the lower edge and

decreases towards the upper. The wider the bridge the more obvious this is, but in a very narrow bridge it may not be detected at all. The lateral spread in the shoot below the wound is always more extensive on that side below the acute angle formed by the lower edge of the wound and the bridge. In the bridge itself reorientation of all the elements, including the medullary rays, to run in the same direction as the bridge, takes place rather quickly, and may be complete at the end of five weeks. Below the bridge, however, reorientation is much slower, and at the end of eight weeks is still more or less confined to the vessels as a whole, the vessel segments as individual units and all the other elements still running practically longitudinally, in the same manner that obtains below the wound with a longitudinal bridge. A similar state of affairs is to be found in the bridge itself during the earlier stages of development (two to three weeks). In sections through the bridge and transverse to the shoot, in material that has been developing for a longer period of time (five to eight weeks), it is found that the elements of the first-formed xylem are all cut transversely and that the later-formed elements are cut progressively more and more obliquely until reorientation is complete. Here again the first expression of reorientation is to be found in the process of vessel formation from a number of segments in obliquely basipetal series. In contrast with the longitudinal bridge it is not infrequently found that there is a greater radial width of new wood within the oblique bridge than occurs at any point in the shoot below it, particularly if the bridge is rather narrow.

(d) *The Zig-zag Bridge (Czapek's ring)*

Development at an early stage, 10-14 days, in relation to this type of wound is depicted in Fig. 6. The first signs of cambial activity may be observed about the same time, just above the upper margin of the wound and in the horizontal part of the bridge exclusive of that area which is common to the lower vertical strip. Within the upper vertical strip cambial activity is basipetal and essentially similar to that in the simple longitudinal bridge. Notice that an obliquely basipetal development from the base of the upper vertical part of the bridge into the horizontal portion is shown. This may not be obvious if the bridge is very narrow. In the lower vertical strip, cambial activity is for the most part basipetal, but towards the distal end there is also a definite obliquely basipetal spread across the bridge from the horizontal strip to the farther margin of the vertical strip. Below the wound an obliquely basipetal development on both sides is again found, and during this earlier stage development is often rather more marked on

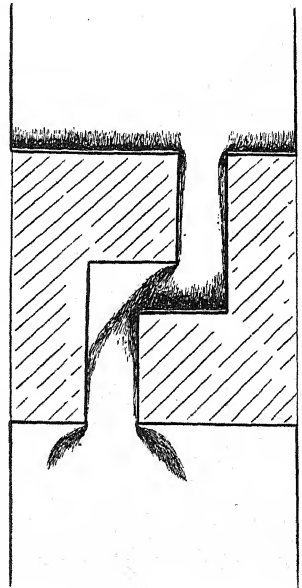


FIG. 6. Local cambial activity in relation to Czapek's ring (zig-zag bridge) expressed in terms of xylem formation.

one side, *viz.*, that side directly below the horizontal portion of the bridge. Otherwise the situation below the wound is exactly the same as that obtaining below the simple longitudinal bridge, and again it is usual to find relatively more cambial activity in the obliquely basipetal spread below the wound than occurs in the basipetal development down the vertical portions of the bridge.

A later stage in development, after four or five weeks, is shown in Fig. 7. There is a marked "piling up" of new xylem, not only above the upper margin of the wound, but also in the horizontal part of the bridge. Usually there is no appreciable difference in the amount of cambial activity in the upper and lower vertical strips. Provided the bridge is fairly narrow, the obliquely basipetal development below the wound may ultimately be practically equal on both sides, but if the bridge is sufficiently wide the greater spread on one side may be maintained.

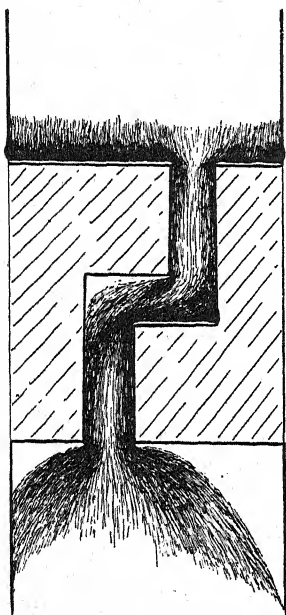


FIG. 7. Same as Fig. 6, but showing more extensive development.

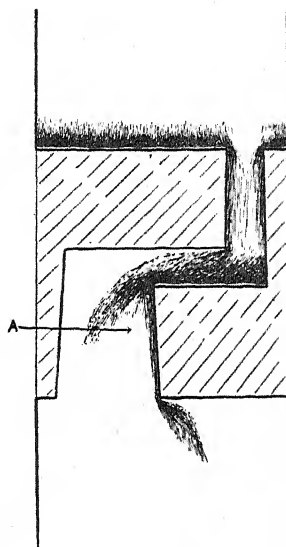


FIG. 8. Local cambial activity in relation to a simple modification of Czapek's ring, in which the lower vertical strip of the zig-zag bridge is made quite wide.

Fig. 8 shows a simple modification of the zig-zag bridge, in which the lower vertical strip is very wide. In such a wound the obliquely basipetal spread of cambial activity from the horizontal strip across the lower vertical strip, and the basipetal spread down the nearer longitudinal margin stand out as two distinct developments, diverging from one another so as to leave an area (A) in which there is no cambial activity. The diagram was drawn from a specimen, three weeks after the beginning of the experiment. After a longer period of time the obliquely basipetal development may ultimately

reach the farther margin of the lower vertical strip and travel basipetally down it, just as in the narrower zig-zag bridge depicted in Fig. 6. The phenomenon just described is of great significance and will be enlarged upon later.

The structural characteristics of the basifugal development of xylem above the wound, of the basipetal development in the two vertical strips and of the obliquely basipetal spread below the wound are exactly similar to those obtaining in the same relative positions in the wound with the longitudinal bridge of bark, and no further comment is called for in this connection. Within the horizontal strip of the bridge, all the elements of the first-formed xylem retain their normal orientation in the longitudinal direction, but the vessels arise from a number of segments in lateral or obliquely lateral series. As development proceeds, the situation becomes quite complex within the horizontal strip, involving much twisting and turning of the elements, and resulting in a very complicated grain of the wood. Even after as long as eight weeks, complete and uniform reorientation of the elements in the transverse direction was not observed, in this material, but it does occur ultimately in a similar wound in the actively growing tree.

Tupper-Carey (27) has followed in detail the structural changes involved in reorientation of the tissues within the bridge in Czapek's ring. Her conclusions need not be discussed here, but she does not stress at all the process of vessel formation as an important factor in this reorientation. In view of the observations, made in this present investigation, on the process of vessel formation from a number of cells in obliquely basipetal series (Plate I, Fig. 5), within the oblique bridge and below all three bridged wounds, the writer is strongly inclined to attribute to this process an important role in the ultimate reorientation of the tissues as a whole, and is also of the opinion that the details might be studied more conveniently, and certainly with less complications, in the oblique bridge than in the zig-zag bridge. These observations lend some measure of support to a former suggestion by the writer (1), that reorientation of the tissues in the form of a flow-pattern, below the sucker bud in poplar roots, may be linked up with the process of vessel formation.

Quantitative Experiments

A large number of shoots were treated as follows. Towards the distal end a complete ring was made, and all buds and lateral branches below the ring removed. Some distance below the ring, for example two inches, a definite wound was made, and then another complete ring at, let us say, four inches below that wound. Finally another wound, exactly similar in shape and size to that between the two complete rings, was made at a much greater distance, for example ten inches, below the second complete ring. The shoot was cut through four inches below this wound and then set up vertically with its basal end immersed in about one inch of water. The definite wounds referred to might be of any type. Actually a circular wound made by means of a sharp cork borer, and bridged rings were used extensively.

The exposed xylem was thoroughly scraped to ensure destruction of the cambium, and the wounds were vaselined in the usual way. The distances between the wounds and the complete rings above varied according to the material, but the situation was always this, that the shoot bore two similar wounds, one of which had a short length of bark distal to it, and the other a much longer length of bark distal to it. In some the shorter unit was distal on the shoot, and in others the longer. On examination after four or five weeks it was invariably found that there was more cambial activity (new xylem) around the wound with the greater length of bark distal to it.

The same relation between the amount of cambial activity around a wound and the amount of bark distal to the wound was also expressed clearly and invariably in another type of experiment (Plate I, Fig. 1). In the particular experiment illustrated the arrangement was as follows. Unit C had a complete cylinder of bark, seven inches in length, distal to the longitudinal bridge. Unit B had the same length of bark distal to the bridge, but the area of bark was much reduced by removal of about half the cylinder, and in the third unit A about three-quarters the cylinder of bark was removed, so that only about one-quarter remained in the form of a long narrow strip seven inches in length. The three units were all located on the same shoot in three-year-old wood, and within the limits of a single year's extension growth. Unit A was the most distally situated on the shoot, and unit C was distal to unit B. In other similar experiments the relative positions of these units to one another were varied. The result of such an experiment is expressed clearly in the photograph (Plate I, Fig. 1), which shows the extent of xylem formation, as revealed when the bark was stripped off after $4\frac{1}{2}$ weeks. Here again it is evident that the more bark there is distal to a wound, the greater is the extent of development of cambial activity in relation to the wound. It does not follow that the width of the transverse cut as such influences in any way the amount of cambial activity, but it does indicate lateral transportation of the stimulus to cambial activity.

Still another experiment, leading to the same conclusions with regard to the proportional relation between wound cambial activity and the amount of bark distal to the wound, and also to lateral translocation of the stimulus to cambial activity, is depicted in Fig. 9. Here a spiral tongue of bark was left running distally from the lower margin of a very wide ring. It was observed that xylem formation increased gradually towards the base of the spiral tongue.

Experiments were also performed to determine the effect of developing buds and leaves within the unit. Main shoots of the same age were selected in pairs, the members of each pair being as uniform in all respects as could be determined by external examination. In one of each pair, a definite wound was made, and the shoot completely ringed some considerable distance, at least 12 inches, distal to the wound. The unit was completely disbudded below the complete ring. The other member of the pair was cut so that approximately the same amount of bark was left distal to the wound but a

few buds, usually two or three, were allowed to remain and develop towards the distal end. Only the distally situated buds were left, because it was desired to observe their effect upon wound cambial activity before the normal basipetal gradient of cambial activity from the developing buds and leaves had reached the level of the wound. The experimental units were therefore of two types, those bearing buds and those without buds. The shoots composing the latter did of course bear buds above the complete ring distal to the wound, but these buds were without the experimental unit as such. Many types of wound were employed, but for microscopic examination the longitudinally bridged wound is perhaps the most convenient.

The leaves began to emerge from the buds after about two weeks, and the material was examined at intervals later. In this way definite evidence was obtained, in several series of experiments, that the presence of developing buds and leaves within the unit increased the amount of cambial activity in relation to the wound, *and this effect was observed undoubtedly before the normal basipetal gradient of cambial activity emanating from the developing extension growth had reached the wound.* The clearest results were obtained four to six weeks after the beginning of the experiment, *i.e.*, two to four weeks after emergence of the leaves from the buds. Not only was there a definite increase in the amount of cambial activity around the wound, but lignification of the new xylem was also more marked. There was also a tendency, not invariable, however, for the vessels to be wider when developing buds and leaves were present on the unit. The above effects were not observed until after the buds had opened and the leaves had developed to a considerable extent, which would indicate that the increase in wound cambial activity is to be related not simply to the presence of buds within the unit, but rather to the development of leaves from these buds.

Table I, showing the number of vessels at certain points in relation to a wound in 14 pairs of contrasting units, with and without developing buds and leaves, gives some idea of the results obtained. In the first four pairs, a small rectangular piece of bark was cut out on March 30, the length of

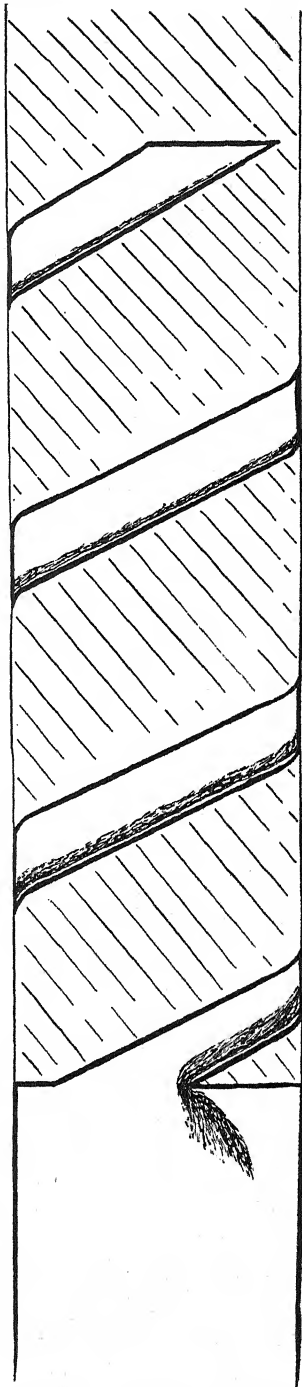


FIG. 9. Cambial activity, expressed in terms of xylem formation, in relation to a spiral tongue of bark running distally up the shoot from a transverse margin.

bark distal to the wound was 12 inches in all cases, and the experiment ran until May 2. The number of vessels that had developed down the longitudinal margins of the wounds was counted from transverse sections. In the next six pairs of units, the wound employed was Czapek's ring, there

TABLE I
NUMBERS OF VESSELS IN CONTRASTING PAIRS OF UNITS, WITH AND WITHOUT DEVELOPING BUDS AND LEAVES

Without buds	With buds
42	87
40	76
25	98
26	53
69	104
80	144
28	65
154	234
67	113
88	217
101	265
173	264
178	324
231	327

were 12 inches of bark above the wound in all cases, and the experiment ran from March 30 until May 3. The figures given are the numbers of vessels in transverse sections of the lower vertical strip of the bridge. In the last four pairs a circular wound was employed, there were 20 inches of bark distal to the wounds, and the experiment ran from April 6 until May 7. The vessels were counted in sections cut through the diameter of the wound and transverse to the unit.

In none of the material considered in the foregoing table had the normal basipetal gradient of cambial activity reached the wound in units bearing developing buds and leaves.

The effect of developing buds and leaves is illustrated quite typically, in two photomicrographs (Plate I, Figs. 4 and 6) of sections from a contrasting pair of units in another experiment. In this case the wound employed was

the longitudinally bridged ring. There were 19 inches of bark distal to the wound in both units, and the experiment ran from April 18 until May 27. The sections photographed were obtained from corresponding positions within the longitudinal bridge in both units. Plate I, Fig. 4 shows the extent of development in the unit in which the two most distally situated buds were allowed to develop, and Plate I, Fig. 6 illustrates the corresponding development in the completely disbudded unit. In this particular case the normal basipetal gradient of cambial activity faded out about three inches above the wound, in the unit bearing developing extension growth.

Sledge (20) reports that he found no difference in the amount of cambial activity at the base of disbudded cuttings relative to control cuttings, after a period of one month. The present writer found the complete ring a very unsatisfactory type of wound to use in quantitative experiments, on account of the "piling up" of cambial activity above the ring, which made it extremely difficult to detect any but the most obvious differences in the extent of development, even when the material was examined microscopically. A similar "piling up" of cambial activity also takes place at the base of woody cuttings, where the conditions are essentially similar to those above a complete ring.

The Effect of Gravity upon Wound Cambial Activity

Experimental units were completely ringed towards the distal end, and all buds and laterals below the ring removed. Some considerable distance, at least 12 inches, below this ring a modified ring was made, in which two longitudinal bridges on opposite sides of the shoot were left connecting the proximal and distal margins. Another complete ring was then made about six inches below this wound, and the shoot cut through approximately six inches below this second complete ring. In other units, two circular wounds on opposite sides of the shoot were often employed instead of the doubly bridged ring. The shoots were arranged practically horizontally with their basal end in water in a very simple manner, *viz.*, by inserting them into ordinary cylindrical metal cans, about five inches in diameter and with an inwardly projecting rim, so that the basal end lay under the rim, and the shoot rested upon the opposite edge. If the cans were filled with water, two shoots pointing in opposite directions could be conveniently inserted in this manner, and a very stable arrangement resulted. The units were arranged so that the longitudinal bridges, or the circular wounds, lay one directly above the other, on opposite sides of the shoot.

After about one month, material that had been kept in complete darkness during the period of the experiment, was examined for local wound cambial activity, when a marked response to gravity was clearly revealed. There was always distinctly more cambial activity on the upper than on the lower side. This response was evident not only in relation to the doubly bridged wound and the circular wounds, but also just distal to the lower complete ring. It was also observed that in many cases the preformed root initials on the upper side had undergone marked swelling, where they occurred along practically the full length of the unit, whereas no such activity was evident on the lower side. Plate I, Fig. 2 shows clearly, in longitudinal and transverse section, the marked difference in the amount of new xylem on the upper and lower side, immediately distal to the lower complete ring in two three-year-old units which had been developing in the horizontal position from May 13 until June 10, in complete darkness. The same marked response was obtained in material developing horizontally in diffuse daylight where the upper side received more light than the lower, but the response is clearly one to gravity and not to light. Actually a number of experiments, which need not be described here, were performed to test the action of diffuse daylight, and no evidence of a light effect on the amount of wound cambial activity was obtained. The effect of gravity was still quite marked in units lying at an angle of 45° to the horizontal.

In another experiment, horizontally placed shoots were rotated through an angle of 180° once every 24 hours for one month, and at the end of that time no difference in the amount of wound cambial activity on opposite sides of the unit could be detected.

Similar experiments were also set up to determine the effect of gravity on the normal basipetal development of cambial activity emanating from growing

buds and leaves. In this case lengths of main shoot were trimmed so that only the more distal buds remained. They were placed horizontally with their basal end in water in the manner previously described, and examined about two months later, when it was found that there was distinctly more cambial development on the upper than on the lower side. Priestley and Tong (16) obtained the same response to gravity in vertical shoots of *Acer* and *Fraxinus*, after they had caused them to develop in the horizontal position for some time. Apparently, therefore, normal cambial activity and local wound cambial activity in balsam poplar respond similarly to the force of gravity. No difference in the degree of lignification of the xylem on the upper and lower sides in horizontally placed units was detected, either with respect to normal cambial activity or to local wound cambial activity.

It will be recalled that a very feeble basipetal gradient of cambial activity, in which vessels and fibres do not occur, arises from the lower margin of a complete ring, but no difference in the extent of development on the upper and lower side in horizontally placed units could be detected.

Discussion and Interpretation of Results

(a) *Quantitative Results*

Snow (23) has very recently succeeded in promoting apparently normal cambial activity in shoots by means of pure auxin- α and hetero-auxin (β -indolyl acetic acid), thus confirming his earlier conclusion (22), that the stimulus emanating from the developing leaves to activate the cambium in the stem below is of the nature of a hormone. In the opinion of the present writer, hormone action appears to supply the most reasonable explanation of the quantitative results obtained in the foregoing experiments on cambial activity in relation to wounding. This suggestion is based upon the following considerations. A definite quantitative relation has been established between the amount of cambial activity in the vicinity of a wound and the amount of bark distal to the wound. This result would be readily understood in terms of the downward movement of a hormone present in the bark. It has also been shown that wound cambial activity is further promoted by the presence of developing leaves distal to the wound, an effect which can be detected before the normal basipetal development of cambial activity emanating from these leaves has reached the wound. This indicates that developing leaves are a source of the hormone promoting wound cambial activity, which suggests further that this hormone is probably the same as that emanating from leaves to promote normal cambial activity. In terms of this suggestion, it is implied that the hormone moves to some extent in advance of the normal basipetal development of cambial activity, which is indeed not at all unlikely. That the hormones promoting normal cambial activity and wound cambial activity are probably one and the same is also supported by the response of these two processes to the influence of gravity. In both cases, development is greater on the upper than on the lower side. Finally, cambial activity in relation to wounding, apart from that obtaining at the

lower edge of a complete ring, is not so very different from normal cambial activity. The same types of elements are produced in both cases, at all events so far as the xylem is concerned, and presumably in the phloem also. If a hormone is involved in normal cambial activity, it would therefore appear reasonable to suspect hormone action in relation to wound cambial activity, and any evidence in that direction assumes significance.

In order to explain the response of both normal and wound cambial activity to gravity, it would appear to be necessary to postulate accumulation of the hormone on the upper side of horizontally placed shoots. There arises in this connection, quite independently of wound cambial activity, a very interesting problem which has not hitherto been stressed. Snow (23) has indicated that cambial activity and cell extension in stems are probably promoted, under natural conditions, by the same growth hormone, *viz.*, auxin-*a*. According to the well known Cholodny-Went theory of geotropism, the growth hormone accumulates on the lower side of plants placed in the horizontal position, and this has indeed been shown to be true experimentally. On the other hand the cambial hormone, which according to Snow is in all probability identical with the growth hormone, must apparently accumulate on the upper side of leader shoots of woody dicotyledons when they are placed horizontally. However the new extension growth of horizontally placed leader shoots of woody dicotyledons is definitely negatively geotropic, so that if the cambial hormone and the growth hormone promoting cell extension and geotropic curvature are identical, we are forced to conceive of the hormone accumulating on the lower side in the region of negatively geotropic curvature, in terms of the Cholodny-Went hypothesis, and then on the upper side in older portions of the shoot in order to explain the observed relation between cambial activity and gravity. This is but one of many paradoxes to be found in the field of plant hormones, which are, however, well worth stressing, if only in order to offset any tendency towards acceptance of an as yet unwarranted simple relation between plant hormones and the processes with which they are connected.

Sledge (20) has suggested that a wound stimulus, functioning through the injection with sap of the intercellular spaces in the vicinity of the cambium, plays an important part in the initiation of basifugal cambial activity at the base of woody cuttings. He then goes on to say that the absence of cambial activity at the distal end of cuttings, where presumably the same conditions of sap injection must hold, and which cannot always be accounted for by a basipetal transport of food, points to the existence of another "unknown factor" which is also involved. It has been shown in the present investigation that a very limited amount of basipetal cambial activity does arise from the lower edge of a complete ring in balsam poplar. However, it would still appear to be necessary to postulate Sledge's "unknown factor" in order to explain, not only the great difference in the amount of cambial activity just above and below a complete ring in completely disbudded units, but in addition, the marked contrast in differentiation subsequent to cell division

in the two cases. In terms of the suggested explanation of the quantitative results obtained in this present investigation, the "unknown factor" is now interpreted as being a hormone.

The question of food supply as a possible alternative to the hormone hypothesis calls for a certain amount of discussion. Sledge (20) came to the conclusion that polarization of wound cambial activity in woody cuttings could not be accounted for as the result of a basipetal movement of food material. The same opinion with regard to normal cambial activity is shared by Snow (21, 22, 23) and others. Loomis (12) found that the relation between active leaves and cambial activity in the shoot below could not be correlated with the production of carbohydrates by the leaves, since carbohydrate levels were higher in sprouting segments which showed but little cambial activity, than in leafy segments where cambial activity was marked. Then again, Priestley and Tong (16) found that, although there was definitely more starch on the upper side of horizontal branches of *Tilia* and *Acer* showing marked epitrophy (*i.e.*, greater radial growth on the upper relative to the lower side), in *Crataegus* which also shows marked epitrophy of its horizontal branches, starch was deposited more heavily on the lower side. In the present investigation no special effort was made to follow the movement and distribution of starch, but it was observed that there was definitely less starch in units bearing developing buds and leaves than in contrasting units which had been disbudded, whereas wound cambial activity was more marked in the former. It is of interest in this connection to note that Loomis (12) has shown that during the earlier development of leaves and sprouts from buds, the movement of food materials tends to be *upwards*. On the whole, therefore, it would appear that a more satisfactory explanation of the quantitative results obtained in this work is to be found in terms of hormone action, rather than food supply.

The marked swelling of preformed root initials on the upper side only of horizontally placed units of balsam poplar is of more than passing interest. It has been shown by Thimann and Koepfli (26) and also by Cooper (5), that root formation is promoted by the same growth hormones that bring about cell extension in stems, and Laibach (11) reports that hetero-auxin promotes both the development of roots and callus tissue in stems. In other words the same growth hormones promote cell extension, root formation and cambial activity, as well as a number of other processes which need not be detailed here. We have therefore the interesting correlation between stimulation of root initials and the greater development of wound cambial activity on the upper side of horizontally placed shoots, which does in a measure support the writer's suggestion that the hormone promoting normal cambial activity is also involved in the development of local wound cambial activity.

(b) *Qualitative Results*

It will probably be admitted that the observed behavior of the cambium in relation to wounding cannot be explained satisfactorily, simply in terms of the accumulation or concentration of a hormone moving basipetally down

the shoot. Consider for example the longitudinally bridged wound (Figs. 2 and 3). In this case the hormone will tend to accumulate above the upper transverse margin, but the experiment illustrated in Plate I, Fig. 1 indicates that lateral translocation takes place quite readily. One would therefore expect to find a greater concentration of hormone within the bridge than just below it. Nevertheless it has been shown definitely that there is relatively more cambial activity in the obliquely basipetal development below the wound, than occurs within the bridge itself. In other words, the amount of cambial activity is not wholly determined by the concentration of hormone. Then again, consider the absence of cambial activity in the region A (Figs. 2 and 8), and the occurrence of cambial activity lateral to it, where presumably the concentration of hormone is lower. Apparently therefore, some other factor plays a part, something no doubt of the nature of a wound stimulus.

At this point the author would like to stress particularly the fact that extreme care was taken in the making of wounds, in relation to which critical observations of the type to be discussed in this section were obtained. The importance pertaining to discontinuity of the elements of the bark as a result of transverse incisions was early recognized, and special care was taken to avoid cutting into the bridge itself, particularly at the basal end. Moreover a common practice employed, which is indicated in many of the figures, was to make longitudinal bridges and the like slightly wider at the basal end than at the distal end. In a word, the writer is satisfied that the observations to be discussed now are truly significant and not the result of imperfect technique, which as a matter of fact is entirely precluded in certain developmental relations showing definite homologies with other cases into which the question of technique might conceivably enter.

The conception of a wound stimulus in relation to wound cambial activity is not without precedent. Sledge (20) postulates a wound stimulus in his work, and conceives of it as functioning through the injection with sap of the intercellular spaces in the vicinity of the wound. Such an explanation is not, however, in the opinion of the present writer, sufficient to explain the observed behavior. It would imply less sap injection within the bridge than below it, which might be possible, but it would not explain the absence of cambial activity at the point A (Fig. 2), where presumably the same conditions of sap injection must exist as in the region lateral to it where cambial activity does occur. Similarly it could not explain the behavior depicted in Fig. 8.

Only one attempt has ever been made hitherto to explain development below bridged wounds and the like, and it is to be found in the work of Janse (10). This investigator was interested primarily in the following question. Does there exist in the plant a force or action which has the tendency to drive or push substances always and invariably in a given direction, ordinarily downwards? As the result of a series of wounding experiments, he came to the conclusion that there was such a force and he called it the "force spéciale," a term which he ultimately replaces by "impulsion basipétale." He observed

the obliquely basipetal development of cambial activity below bridged wounds, and he interpreted this phenomenon as the result of the action of two other forces, *viz.*, the "attraction du cambium" and the "attraction des tissus traumatiques," upon his third force, the "force spéciale" or "impulsion basipétale." Let us now consider a particular case, for example, development below a rectangular wound of the type depicted in Fig. 4. Janse's interpretation would be as follows. Below the lower transverse margin of the wound two forces are to be considered, (i) the "attraction du cambium" which acts in a horizontal direction simply because the margin of the wound is transverse (if the wound margin were oblique the direction of this force would also be oblique, according to Janse), and (ii) the "attraction des tissus traumatiques" which acts perpendicularly to the wound margin. These two forces set up, in the region below the wound, a so-called field of attraction which reacts upon the "impulsion basipétale" acting longitudinally from above downwards, in such a way as to cause lateral diversion of the "impulsion basipétale" below the wound. As a result of this, food material is also diverted laterally in this region to nourish the cambium in its path. He does however write as follows: "Il faut se rappeler cependant ici que l'afflux de nourriture ne doit être considéré comme la cause du développement des tissus, mais que c'est au contraire la division des cellules et leur agrandissement qui sont les causes du courant qui va se diriger vers elles." But nevertheless throughout his paper he clearly considers food supply to be the important factor in development, subsequent to the change in direction of the "impulsion basipétale."

Janse's interpretation is decidedly obscure, if only by dint of lack of definition of his forces. His "impulsion basipétale" may be interpreted as being simply the recognized tendency for cambial activity to develop normally in the basipetal direction, and it is generally conceded that the direction may be obliquely basipetal without necessarily postulating the action of contributory forces. He stresses greatly the action of the "impulsion basipétale" upon the downward movement of food materials, whereas the same reaction of the cambium can be observed in relation to wounds in regions where the general movement of food materials is upwards, *e.g.*, in regions bearing developing buds or sprouts (*c.f.* Loomis (12)). Then again it is by no means clear how Janse would explain the absence of cambial activity in the region A (Figs. 2 and 8) in terms of the interaction of his three forces.

The present writer is also of the opinion that a wound stimulus is involved in cambial activity in relation to wounding, and ventures to suggest that a satisfactory explanation is to be found in the conception of this wound stimulus in terms of a definite wound substance. This hypothesis is based upon the observations made in this investigation and upon certain very significant correlations. It will first of all be stated briefly. The main points are as follows:

- (1) Subsequent to wounding, a definite wound substance is produced as a result of death or dying of the cells bordering upon the wound.

(2) The amount of this wound substance produced is proportional to the extent of dying.

(3) The wound substance can of itself promote cell division only, in the cambial layers.

(4) Differentiation of more or less typical xylem and phloem, and in addition further cell division, is promoted by the cambial hormone which moves basipetally down the living bark and reacts with the wound substance to give the observed behavior.

In the discussion to follow, the writer proposes at times to discuss his observations in terms of the hypothesis, and at other times the hypothesis will be discussed in terms of the observations and correlations which led to its formulation. In terms of the hypothesis the marked contrast in local cambial activity at the upper and lower margins of a complete ring is readily understandable. Development at the lower margin is to be attributed almost solely to the action of the wound substance, causing cell division only in the cambial layers. The absence of differentiation (with the exception of a few tracheids) to form vessels and fibres is the result of depletion in this region of the cambial hormone, on account of its basipetal transport down the shoot. Cambial activity involving differentiation of xylem just above a complete ring is the result of interaction between the wound substance and the cambial hormone which moves basipetally towards the wound margin from above. However the difference between cambial activity at the upper and lower margins of a complete ring is not simply the presence of vessels and fibres in the one case and their absence in the other. It is clear that the cambium divides more frequently above a complete ring than below it, so that one has to conceive of the cambial hormone as promoting not only differentiation of xylem elements, but also, as might indeed be expected, further cell division.

Consider now behavior in relation to the type of wound depicted in Fig. 1, in which a longitudinal tongue of bark runs distally from the lower margin of a complete ring. Here again there is little or no differentiation of vessels and fibres, on account of basipetal transport of the cambial hormone. The interesting points are that stimulation of cambial activity is greater longitudinally from the transverse margins than laterally from the longitudinal margins, and the exceedingly suggestive correlation that dying of the cells subsequent to wounding is also more extensive longitudinally from the transverse margins than laterally from the longitudinal margins. Hence the hypothesis to the effect that production of wound substance is proportional to dying subsequent to wounding.

It has been shown that there is definitely more cambial activity above and below a longitudinally bridged ring than occurs within the bridge itself (Figs. 2 and 3). The correlation that immediately suggests itself here is that dying subsequent to wounding is more extensive longitudinally from the upper and lower transverse margins of the wound than laterally from the longitudinal margins, which implies that there will be more wound substance produced

just above and below the wound than within the longitudinal bridge. On this basis therefore, it is suggested that the observed behavior might be interpreted as the result of a low concentration of wound substance within the bridge acting as a limiting factor, with the proviso that the limiting action is relative, not absolute. Precisely the same interpretation can be applied to behavior in relation to Czapek's ring (Figs. 6 and 7), where again there is relatively more cambial activity in the obliquely basipetal development below the wound than occurs within the vertical strips of the zig-zag bridge. On the other hand the condition just discussed is not apparent in relation to the obliquely bridged ring, which is just what might be expected, since dying longitudinally from a definitely oblique wound margin is of the same order as that obtaining from a transverse margin. One would not therefore expect to find wound substance limiting within the oblique bridge.

It still remains to explain a peculiar feature of the obliquely basipetal development below bridged wounds and the like. Let us consider specifically the longitudinally bridged wound (Fig. 2), although behavior below all bridged wounds is essentially similar. Below the lower transverse margin of the wound there is depletion of the cambial hormone as a result of its basipetal transport, similar to that obtaining below a complete ring. However there is still a supply of this hormone arriving from above by way of the longitudinal bridge, below which it spreads out obliquely basipetally on both sides from the region of relatively high concentration in the same longitudinal line as the bridge into the depleted region below the lower transverse margin of the wound. The question then arises, why is there no cambial activity in the region A (Fig. 2), whereas cambial activity occurs lateral to it, where presumably, the concentration of cambial hormone must be lower. In order to explain this, the writer suggests that the wound substance emanating from the lower transverse wound margin is used up when it reaches and interacts with the cambial hormone, at what might for convenience be termed the "diffusion" front of the cambial hormone, in terms of which, the absence of cambial activity in region A is again to be interpreted as the result of lack or low concentration of wound substance acting as a limiting factor. At points lateral to A, where cambial activity does occur but which are equally far removed from the lower transverse margin of the wound as A, wound substance has not been limiting, simply because it has been free to move down that far before meeting and interacting with the cambial hormone at its obliquely basipetal "diffusion" front.

The exceedingly interesting behavior observed in the modified zig-zag bridge (Fig. 8) can also be readily interpreted along the same lines, in the following manner. The obliquely basipetal development of cambial activity across the lower vertical strip is the result of interaction between the wound substance emanating from the transverse margin just about it and the cambial hormone at its obliquely basipetal "diffusion" front, in exactly the same manner that obtains below the bridge in the same specimen. The basipetal development down the shorter longitudinal margin of the lower vertical strip is similar

to that within a longitudinal bridge, and the absence of cambial activity in the region A is again due to a low concentration of wound substance acting as a limiting factor. There would seem to be little doubt in this case but that the concentration of cambial hormone must be higher in region A than at points lateral to it on the left, where cambial activity does occur. It is suggested that the wound substance emanating from the transverse margin of the lower vertical strip is used up in interaction with the cambial hormone just above and lateral to the left of region A, and there would seem to be little possibility of wound substance reaching this region from the longitudinal margin to the right, since what little of it is produced there (*c.f.* longitudinally bridged wound) is used up in the basipetal development of cambial activity down that margin. To summarize then, local cambial activity in relation to bridged wounds and modifications of such is to be interpreted as the result of interaction between a wound substance, produced subsequent to wounding, and the cambial hormone which is present in the living bark and moves basipetally down the shoot. At certain points in relation to a wound, a low concentration of wound substance may act as a limiting factor, but its limiting effect is only relative, since cambial activity is further promoted at all points by increased supplies of the cambial hormone (*c.f.* quantitative experiments).

Although interaction between a wound substance and a hormone is, so far as the writer is aware, a new interpretation of local cambial activity in relation to wounds, the idea is not without precedent with regard to certain other growth processes. Haberlandt (6, 8) found that cell division at the cut surface of potato tubers was most marked in the immediate vicinity of vascular strands. The xylem of the bundle did not seem to play any part, all that was necessary was phloem tissue. Pieces of potato which did not contain any vascular tissue also showed cell division, but not to the same extent as when phloem was included. If a piece without bundles was separated from another piece with bundles by means of a thin film of agar, then cell division at the surface of the former was increased. He came to the conclusion that cell division in pieces of tissue containing vascular bundles was the result of interaction between a hormone produced in the phloem and a wound hormone produced subsequent to wounding. A summary of Haberlandt's work along these lines is also to be found in a paper by Pringsheim (17). Then again, Nakano (13) came to the conclusion that besides correlative influences, the interaction of two hormones was also involved in the production of typical callus tissue. On the other hand, it is of interest to find that Cholodny (3) has attempted to explain certain experimental results by postulating that a wound hormone retarded growth, by neutralization of the effect of the growth hormone.

Haberlandt (7, 8) found that he was able, not in every case however, to limit the amount of cell division subsequent to wounding, by washing the wound surface thoroughly in a stream of water. The present writer performed similar experiments, which need not be described in detail here, with wounded

balsam poplar. The wound surface was washed for 10-15 minutes in a stream of tap water, and then for a few minutes in distilled water. No evidence was obtained that the amount of cambial activity in relation to the wound was in any way affected as a result of washing, and the extent of dying subsequent to wounding was, as far as could be determined, of the same order in washed and unwashed wounds.

In the present paper the writer has refrained from designating the wound substance as a wound hormone, since apparently there is some doubt as to the legitimacy of so doing. Petri (14), in a recent paper, concluded that the mode of origin and properties of so-called wound hormones seemed to preclude of their being so defined, even according to the concept of plant hormones. He has suggested that they be considered as an oxidation product of a compound normally present in living cells.

Before concluding, a few remarks relating to further research on the subject of local cambial activity as a result of wounding might be in order. At the present time much is becoming known about the isolation and purification of plant hormones, and in some cases synthesis has been effected. Snow (23) has succeeded in promoting cambial activity by the use of pure hormones. The response was obtained just below the point of application of the hormone. With regard to wound cambial activity on the other hand, the ideal experiment would be one in which a response is obtained in the immediate vicinity of the wound, as a result of application of the hormone at a point some distance from the wound, and in which the response at the wound is more or less distinct and separate from any development arising at the point of application. Stimulation of root development in regions considerably removed from the point of application of the growth hormone has been obtained by a number of investigators, some of whom have already been mentioned (*c.f.* Laibach, Thimann and Koepfli, and Cooper), but nothing is known about the response of the cambium in these experiments. Should, however, an experiment of the type just outlined be successfully performed, the whole problem of certain other relations, discussed in this paper in terms of interaction between the cambial hormone and a wound substance, would still remain, so that further experimentation is called for in this connection also, in an attempt to determine more definitely the nature of the wound stimulus. The writer himself hopes to be able to continue his experiments along the lines indicated, and it may be that the results obtained in this investigation, and the interpretations suggested are of sufficient interest to stimulate research by other workers.

Acknowledgment

The author desires to acknowledge with thanks his indebtedness to Miss Elizabeth N. Johnson, who not only by her willingness to relieve the author of various routine tasks over a considerable period of time, but also by her understanding of the problem, assisted very considerably during the later stages of this research.

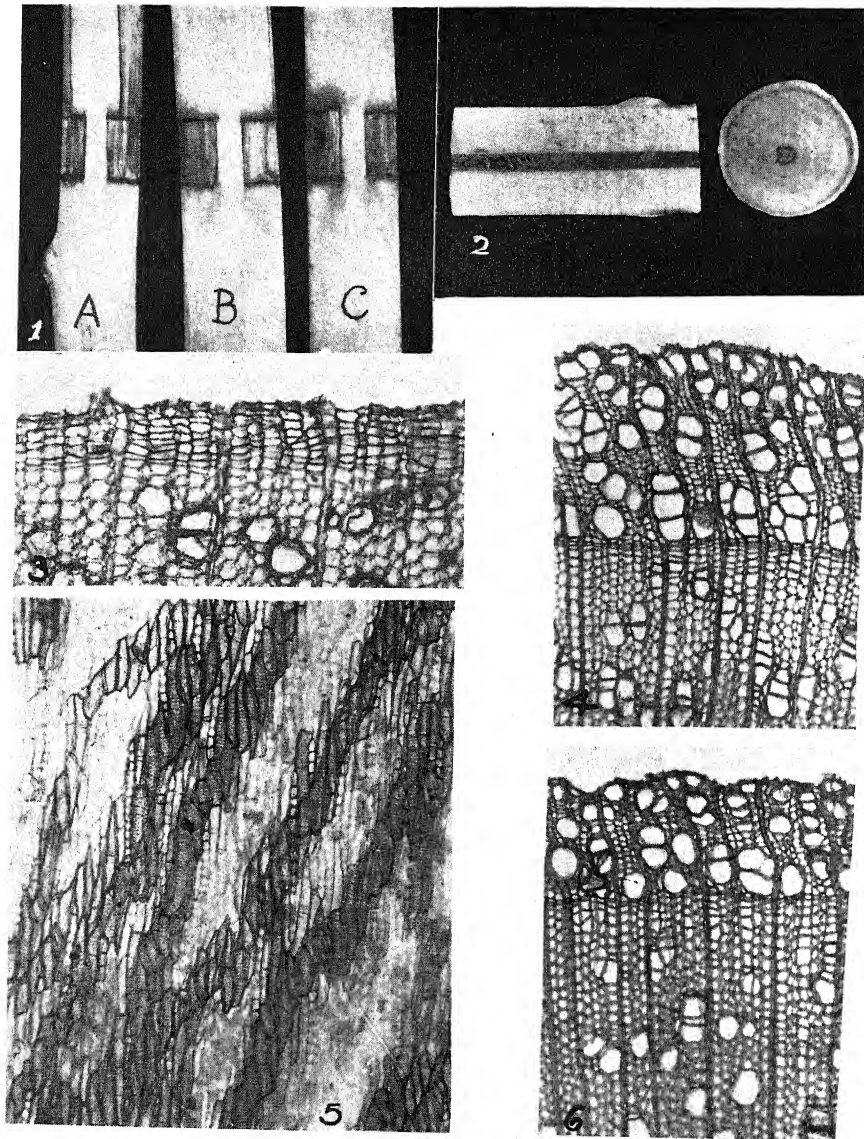


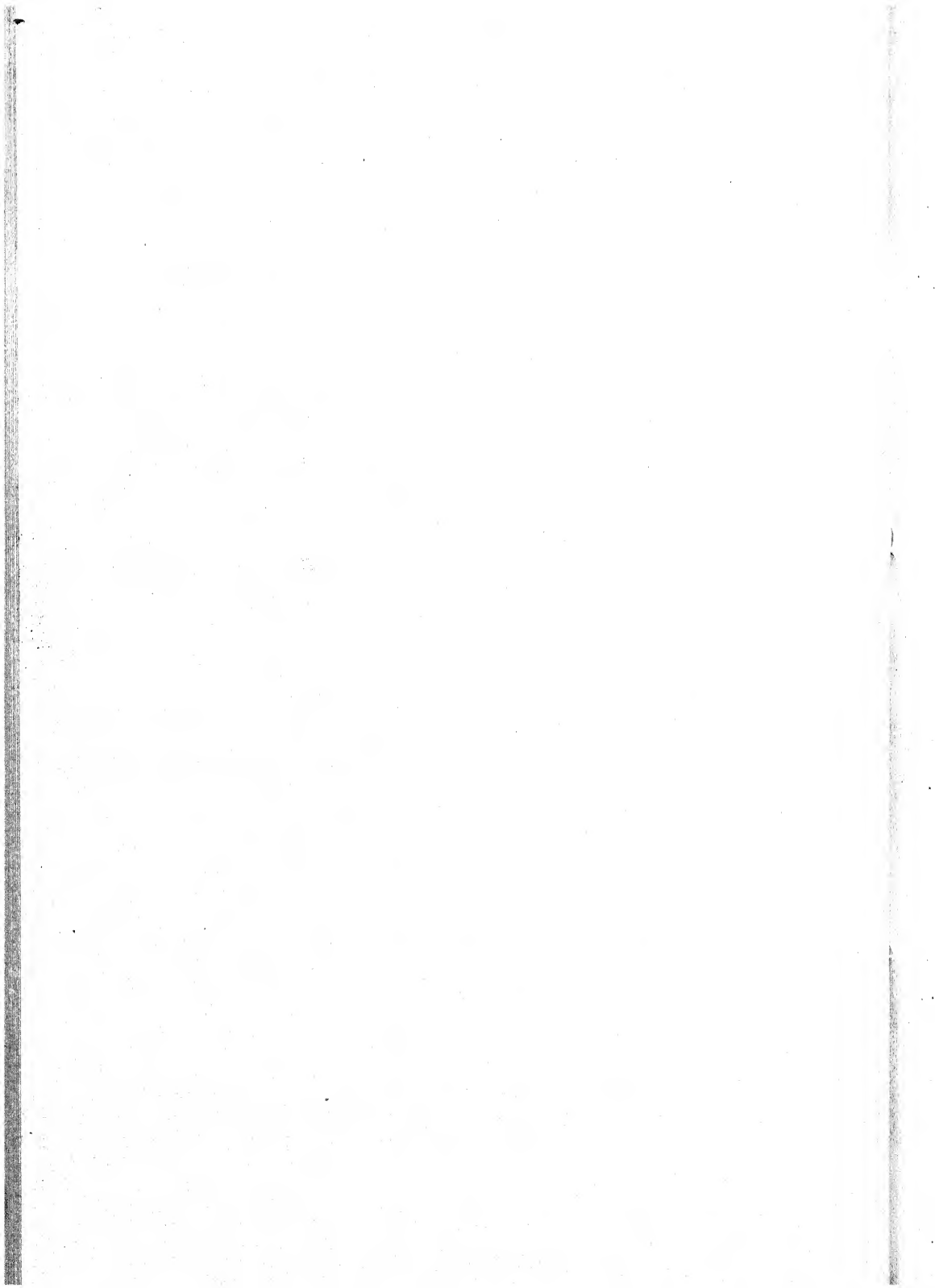
FIG. 1. Three units of balsam poplar in 3-year-old wood obtained from the same leader shoot, illustrating the relation, in terms of xylem formation, between the extent of cambial activity in the vicinity of a wound and the amount of living bark distal to the wound. Four-fifths natural size.

FIG. 2. Illustrating in longitudinal and transverse section, the effect of gravity upon local cambial activity just distal to a complete ring, as revealed when the bark is peeled from the shoot. Cambial activity is more marked on the upper side. X 1.6.

FIG. 3. Illustrating wound cambial activity just below a complete ring. Transverse section of a peeled shoot just below a complete ring, showing several layers of more or less regularly rectangular cells cut off to the inside by the vascular cambium. X 128.

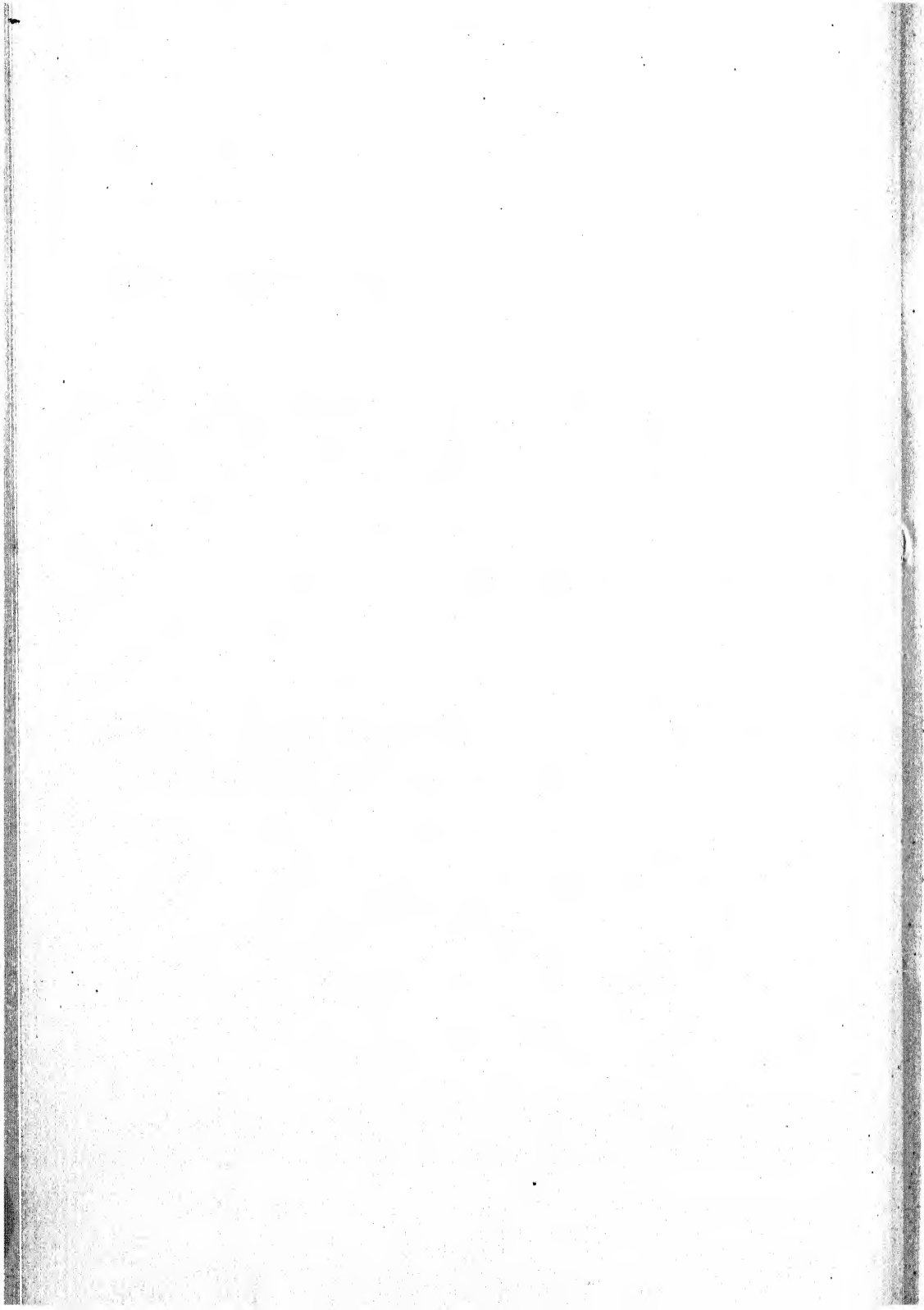
FIGS. 4 and 6. Xylem formation at corresponding points within the bridge of a longitudinally bridged ring in two contrasting units. In one unit (Fig. 4) two buds at the distal end were allowed to develop, whereas the other unit (Fig. 6) was completely disbudded. About one-third of the bridge, cut transversely, is shown in each photograph. X 96.

FIG. 5. A sheet of xylem from the obliquely basipetal development below a bridged wound.



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THE SOIL-BLOCK WASHING METHOD IN QUANTITATIVE ROOT STUDY¹

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Abstract

It has been observed consistently that competition among plants first takes place between the root systems, and that the nature, vigor, extent and distribution of the root systems have an important bearing on the development of top growth. A new technique for root studies, the Soil-block Washing Method, is described in considerable detail. This method enables the investigator to procure entire root systems at any stage of plant development, from plants grown under natural soil conditions.

Introduction

The root systems of plants have received less attention than the above-ground parts, largely because the latter are conspicuous, have definite economic value in many cases, and form an easily available source of material for study. As a result knowledge of root systems was scarce and inaccurate for a long time, and today it is still inadequate.

Since the eighteenth century, many attempts have been made to secure the root systems of various wild and cultivated species in sufficiently sound condition to allow of study of their nature and extent. As a result of such work considerable light has been thrown on a hitherto obscure phase of the plant's development. Now it is well established that under all conditions the root systems perform two distinct functions. The first is essentially physiological in nature. This relates to supplying the plant organisms with food substances from the soil solution or with storage of the food reserves manufactured within the plants. The second is purely mechanical and effects the anchorage of plants in the growing medium (10). If either function or both are disturbed the effect is decrease of the top growth or the ultimate death of the plant. On the other hand, plants with healthy and extensive root systems develop very strong top growth (25) capable of resisting a surprisingly great amount of hardship and injury imposed by such conditions as heavy winds, direct heat, mechanical cutting, grazing, or tramping. Besides these functions, the root systems under all conditions play a leading part in keeping the plant in the best possible balance with the environment.

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Upon germination, for instance, roots are the first visible organs. They also are first to adapt themselves to various soil conditions before the stem successfully emerges above the surface (26). During the plant's life roots must continuously adjust themselves to the changing conditions in the ground, as otherwise the top growth will perish (24). Root systems of various plant species and varieties respond differently toward deep and shallow, heavy and light, dry and moist soils (12, 14, 20, 39). Similar variations are observed with respect to their behavior toward the application of different mineral fertilizers (31). The susceptibility of plants to damage from different root-diseases and insect pests also is governed to a considerable extent by their ability to regenerate the damaged roots or to replace them by new ones (34).

The above considerations, based on abundant experimental evidence, indicate that the root systems are always the first part of the plant to be profoundly influenced by the entire complex of growth factors. Consequently the top growth, which depends directly upon the performing capacity of the roots, is only a true resultant of the interaction between the growth factors in a given habitat and the root systems of the plants subjected to their direct and continuous influence. Therefore it is only logical to expect that adaptation of species and varieties to ecologically different regions depends largely upon the abilities of their roots to thrive under many environmental conditions. If the soil temperature or moisture conditions of a habitat are detrimental to the roots of an introduced species, it will never become established. Plants with roots that barely survive the test usually remain in the district, but only as subdominant vegetation without economic significance. On the other hand, plants with roots well fitted to the new environment thrive, and in time become ecotypes of the habitat. They soon assert their positive (successful crop introductions) or negative (successful weed invaders) influence upon the country in accordance with their economic value.

In view of these facts it was thought that a quantitative study of root systems extricated from their natural environment in the least damaged condition undoubtedly would provide information fundamental to the understanding of the plant's top development. Such information may be of value in such phases of agricultural investigation as soils, weed control, field management, plant ecology, plant breeding, plant pathology and entomology. The results also may prove to be of particular importance in practical work concerning the rehabilitation and reclamation of land by amelioration projects, such as irrigation (12) or the assigning of certain areas to grass production. The reliability of results from a root study, however, depends largely upon the technique used. Therefore an attempt has been made here to give at least a brief systematic review of the literature dealing with the subject as well as to describe and discuss adequately the technique developed in the quantitative root studies at the University of Saskatchewan. In the review of literature the methods have been grouped on the basis of similarity of essential features.

Methods Previously Used in Root Studies

1. *Direct Washing Method*

Hales was perhaps the first to comprehend fully the value of information on the extent of the root systems of agricultural plants from the standpoint of crop production. He made an attempt to obtain root systems of some agricultural plants as early as 1727 (6). His simple method consisted of a direct washing from the ground. Apparently it was a long and awkward procedure, because it found no followers among his contemporaries and was soon abandoned by its originator.

2. *Trench-washing Method*

In 1855 Schubart (29) followed a different procedure. This may be properly called the "Trench-washing Method". He first dug a trench beside the plant or plants selected for the study and then washed out the root systems directly from a vertical wall of the trench. The method enabled him to determine the depth of penetration and to procure in bulk root material of such crops as wheat, winter rape and clover. However, this method does not include means whereby the root systems of individual plants may be separated from the material and studied in detail. The method, therefore, is better adapted to investigating qualities of root material than to quantitative studies of the root systems of individual plants. Schubart's method was followed more or less closely by Goff (4), Morrow and Hunt (21) and others.

3. *Water-culture and Soil-containers Method*

Nobbe (22) was interested in the effects of different mineral salts upon the development of root systems and their structures. He realized the difficulty in carrying out investigations with plants grown in the natural undisturbed ground and used large glass cylinders filled with certain kinds of soil mixtures. He grew the seedlings in water cultures and then transplanted them into the cylinders. At definite stages of plant development the entire content of the cylinders was removed and the root systems liberated from earth by soaking in water and shaking with the fingers. Kraus (16, 17) used pots and wooden boxes for the same purpose. Hoveler (11) and Frank (3) studied corn, peas and beans in vessels filled with various soils. Tucker and von Seelhorst (37) investigated the root systems of oat plants grown in zinc cans containing about 40 lb. of soil. Arker (1) worked with *Lupinus* and sunflower in either water cultures or soil containers. Very extensive study of the root systems of wheat, oats and barley by von Seelhorst and Freckmann (32) was entirely carried out in pots. Mielecki (19) used water cultures exclusively in studying effects of potash on the development of the root systems of different plants.

By growing plants in the various kinds of soil containers and washing the roots with water, or by using water cultures, the workers were in a position to secure root material and sometimes even root systems of single plants in good condition without much effort. The method is cheap, quick and easy. However, the results obtained under such highly artificial conditions, although

of theoretical interest, do not illustrate the usual extent, shape, penetration, branching, and consequently performance, of the root systems, which the latter exert when grown in the natural habitats.

4. *Hellriegel's Steel Cylinder Method*

In 1883 Hellriegel (9) described a very interesting method for studying roots of plants grown in the open fields. He used steel cylinders about 400 sq. cm. in cross section and as long as required by the material studied. The implement was first driven into the ground to the depth desired. Then the earth around it was removed and the base of the soil column inside the cylinder was examined with respect to the number of root stubs found at that level. Von Seelhorst (31) and other workers also used this method in several experiments. The method, however, is suitable only for studying depth of penetration and sometimes the lateral spread of the root systems.

5. *Steel-frame Washing Method*

In studying the root systems of corn plants, Hays (7) used steel frames constructed of water pipes one inch in diameter. At intervals of 2-3 inches in depth, two-inch wire netting was fastened across the frame in order to provide supports for the roots. The frame was set in the ground, filled with sifted soil, and corn planted in the centre of it. At certain stages of growth the frames were excavated and the loose soil washed away, leaving the root system hanging over the wire netting inside the steel structure.

6. *Soil-prism Washing Method*

Following the principles of Hays' method, King (15) developed a procedure, differing only in that the plants were established in the undisturbed soil and grew under natural field conditions until the stage at which they were to be studied. Then a prism of soil one foot thick and as long and deep as desired was dug, and reinforced by a steel or wooden frame very similar to that constructed by Hays. The wire netting was stretched over the four vertical sides of the frame to keep the soil column intact. Many thin rods driven through the prism were substituted for the wire netting used by Hays. The loose dirt from the top of the prism was removed and replaced by a layer of plaster of Paris in order to fix the top growth in a permanent position. Soil from the prism was gradually removed by means of water, washing proceeding from the top down. The root systems thus freed of earth were suspended from the layer of plaster of Paris and held in their relative positions by the cross rods. King's method has been used by Ten Eyck (36), Goff (5) and Shepperd (33). Besides being extremely muddy and slow (as one has to work in the hole flooded with water and thin mud), the method had several other important disadvantages. First, owing to the fact that the washing started from the top of the prism, the younger, weak roots and particularly fine root branches, which are the most important water absorption structures, were usually completely lost, being torn away by clods of soil collapsing under the pressure of the water spray. Second, the prisms usually were too narrow, and therefore the root material obtained from them represented only a cross section through the root systems, rather than the root systems in their entirety.

Otuka (23), in his root study of the apple tree, considerably modified the prism washing method by discarding the steel frame and cross rods used by King. Part of an orchard was fully excavated, a number of soil prisms of definite size and shape being left at intervals over the excavated area. These were then encased in wooden boxes to prevent roots from extending beyond the prism walls. After this the entire excavation was filled in and the surface leveled. Approximately 10 apple seeds were planted in each prism. At certain stages of plant development the soil and wooden boxes around each prism were removed and the earth washed away as in King's method.

7. Concrete-compartment Washing Method

Schulze (30) was interested in extricating entire root systems from the ground. Realizing the difficulty of the task with plants grown in the undisturbed ground, he constructed a number of separate compartments each $24 \times 24 \times 80$ inches in size. Three walls of each compartment were made of concrete, while the fourth side opened into a room common to all compartments. The open side was then closed by two metal plates. The inner plate was perforated with holes about $\frac{3}{8}$ in. in diameter, while the outside one was solid in order to make the compartments water-tight. The compartments were packed with sifted soil and sown to different crops. At various stages of plant growth the root systems were studied in the following manner: A stream of water was used to remove the loose soil, which was carried off through the perforated plate, the solid plate having been taken away before washing started. In spite of constructional differences the method closely resembles that of Hays as in both cases sifted soil was used instead of the natural ground.

8. Nail and Needle Brush Washing Methods

Introduced by Rotmistroff (27) and modified by Maschhaupt (18) and Spirhanzl (35) this method is designed to keep the roots in their original position after the earth is removed by water, and thus to disclose a true picture of their distribution under the surface. The principal features of the method as used by Rotmistroff may be described as follows: a wooden box only one inch wide but about 28 inches long and 60 inches deep was packed with sifted soil and set into the ground. In this narrow box plants grew to a certain stage of development. Then the box was excavated and one side board replaced by a zinc plate filled with one inch nails to form a sort of nail brush. Loose dirt from the box was washed off by a stream of water and roots were left held in place by the nails of the zinc brush. Maschhaupt applied a similar principle but worked with plants grown in the field. The brush in this case consisted of a large board filled with long needles. This was forced against the vertical wall of a trench dug on one side of the plants selected for the study. In addition he used a large steel sheet, which he forced into the ground at the desired distance in front of the needle brush. The washing was done as in Rotmistroff's method.

Spirhanzl constructed a nail brush different from either of those mentioned. Instead of a solid board he used a screen made of wooden slats and drove the nails into it where the slats crossed. Water was run against the wall face to be washed through the square holes between the slats. After a thorough trial this method proved to be unsatisfactory and was discarded. Spirhanzl then used his brush to wash the earth from blocks of soil fully excavated and brought to the surface. In this case the results were more satisfactory, yet it took as much as 13 days of continuous work to remove the dirt from a comparatively small block. He concluded, therefore, that a satisfactory method for root study still remained to be found.

9. Observation-pit Method

Rotmistroff (28) developed also an interesting observational method for root study. It consisted of a pit 27 feet long, 3 feet wide and 4 feet deep, dug along one side of a plot where the penetration of roots was to be observed. In a vertical wall of the pit horizontal holes two inches high, four inches wide and eight inches deep, were made. These were spaced 20 inches apart on the horizontal lines and 4 inches apart on the vertical lines. In the course of penetration, roots met the holes at different depths and their tips projected through the upper walls. These were observed and the depths recorded. To prevent the holes from drying out they were plugged with wooden blocks. The vertical wall of the pit was covered with a thick sheet of asbestos paper and, in addition, the entire pit was protected by boards and thick straw mats.

From time to time the protections were removed and observations made. To determine the lateral spread of roots, similar holes dug vertically at certain intervals from one another have been used, successive holes being dug at a greater distance from the row of plants under observation. It is claimed that the tips of the lateral roots could be easily observed and the greatest spread accurately determined.

10. Direct Tracing of the Main Roots

By the end of the nineteenth century, some workers began to realize that all the attempts so far made to procure, in their entirety, the root systems of plants growing in the undisturbed ground, usually produced incomplete and badly dislocated root material. This suggested that it would be advisable to sacrifice some fine root structures and to study the main roots in their exact positions as observed when they were individually traced in the ground with sharp pointed instruments. In this manner Headden (8), in 1896, examined the root systems of six-year-old alfalfa plants and determined the greatest depth of penetration. Six years later, Cottrell (2) traced the main roots of an eight-year-old alfalfa plant in the ground to a depth of more than 10 feet. The technique used by these two workers, however, was not sufficiently stabilized and although it certainly signalized a definite turning point in the efforts in root study, it can hardly be regarded as a distinct method.

11. Weaver's Method

The principle of tracing roots directly in the ground was also adopted by Weaver of Nebraska in his extensive ecological researches with native and cultivated plant species. He clearly described individual phases of the work, and produced a definite method in root study, which now is generally known as Weaver's trench-tracing method. Weaver (38) has briefly described the method as follows: "The methods employed in excavating root systems was to dig trenches 2 to 3 feet wide and 6 to 10 feet long to a depth of about 6 feet by the side of the plants to be examined. This offered an open face into which one might dig with a hand pick furnished with a cutting edge on one end and, after sufficient practice and acquaintance with the soil texture, successfully excavate a root system almost in its entirety." Owing to the skill and perseverance with which this method has been used by Weaver and his co-workers over a long period of years, it has been the dominant method in root study and is so at the present time.

In Canada Weaver's method has been consistently followed by Simmonds and his co-workers in the study of root-rot disease of wheat (34).

Soil-block Washing Method

1. General Considerations

The objective in root study of any kind should be to determine exactly the underground development with respect to at least one of the following three cardinal points:

(a) The habit of root growth as indicated by the natural spread and course of penetration of roots.

(b) The quantity of root material found at different ground levels.

(c) The performing capacity of root systems as indicated chiefly by the amount, extent, and location of the fine root branches on the main roots of each species.

2. Historical

It is well to keep in mind that the method herein described was developed, not from a special project outlined for the purpose, but as a necessity in work primarily concerned with the competitive efficiencies of different species grown together under ordinary field conditions. In this study it was observed that the performance of the aboveground growth of crop and weed species was always governed by the condition and extent of their root systems. It is obvious, therefore, that in a study of this kind, information concerning the three above-mentioned points is essential. To obtain such information on the root systems of the plants studied, several methods previously used by different workers were tried, but with unsatisfactory results. It became evident that an altogether different process for obtaining representative samples of the root systems was needed. Such a process was eventually found and is described here as the Soil-block Washing Method.

3. *Principles Underlying the Method*

The method was devised to meet the following requirements:

(1) Extrication of root systems of plants grown under ordinary field conditions in various types of soil, with the finest young branches in undamaged condition, at any stage of plant development.

(2) Removal of large quantities of earth from the blocks in a reasonably short time, thus reducing cost and providing an opportunity for comparative study of several plant species at the same time.

(3) Supplying root material in such condition that the root systems of individual plants of various species grown in competition may be separated and studied in detail.

4. *Plant Material*

During the first year of this study, plant material grown both in various soil containers in the greenhouse and in the open field, was extensively used. The results obtained from the two sources had adequately demonstrated that the root systems of the plants, grown even under most favorable greenhouse conditions, never attained more than 60% of the growth attained by the same species under comparatively poor environment in the open field. Considering this fact together with the desirability of procuring results directly applicable to ordinary farming conditions, it was decided in 1931 to discontinue work with plants grown in artificial media.

Experience has shown that practically all cereals and the majority of forage crops as well as annual, biennial and many perennial weed species make excellent material for root study with the soil-block washing method. Only perennials with extensive side runners cannot be studied by this method at the later stages of their development. In spring the selected species are seeded on a piece of land which has been previously treated in the same manner as the ordinary farm fields. Usually a number of single plants and several plots of each crop free from weeds (checks), and in competition with weeds (competition plots), are seeded the same day. Seeding in the check and competition plots is most conveniently done with the Columbia hand drill at ordinary rates of seeding and with rows spaced six inches apart. In the competition plots, weeds are sown between the crop rows with the same drill at the rate desired but much shallower than the cereals and at about the same depth as the forage crop seeds. The single plants are sown each in the centre of a plot ten feet square to eliminate competition of any kind.

5. *Excavations*

To follow the gradual progress in root development of each species from emergence to maturity it is necessary to make excavations of plants that emerged on the same date, at several stages of their development. With annual species it was found that excavations at 5, 22 and 40 days after emergence and one at maturity give a sufficiently complete picture of the entire

process of root increase during their life period. In biennial plants excavations made at 5, 22, 40 and 100 days after emergence, and in the next year one in May and one before maturity were sufficient. Perennial plants should be excavated at the same stages as biennials during the first year and, in addition, once in August of the second year, and again in August of the third year of growth.

6. *Size of Soil Blocks*

The soil blocks are cut in such sizes as to include the entire mass occupied by the root systems at each stage of growth. Dimensions of the blocks vary with the plant material, type of soil, amount and distribution of moisture in the ground, and many other factors. For this reason the sizes of blocks cannot be stated dogmatically for all conditions. Under the conditions prevailing at Saskatoon, blocks $14 \times 14 \times 14$ inches have been large enough to include entire root systems, at the five-days stage, of all the cereal crops studied over a period of six years. Forage crops and weeds invariably take smaller blocks at this particular stage. For 22-day material blocks $24 \times 24 \times 32$ inches have proved to be sufficiently large. At the 40-day stage blocks $26 \times 26 \times 46$ inches have frequently been required. At maturity, cereal crops take blocks



FIG. 1. A block of soil $40 \times 40 \times 70$ inches completely cut out and ready for encasing.

Fig. 2 presents a diagrammatic view of the encasing frame and Fig. 3 illustrates it in use. For small blocks, all sides of the encasing frame are made of one-inch lumber. For blocks weighing over 500 lb. the backs (*m*, Fig. 2) of the frames are constructed of two-inch material. The side-bars (*d*) are made of 2×4 in. material regardless of the size of the block. In all cases where the weight of a block exceeds one ton, the cross bars of the backs (*h*) should be made of heavy steel. The back (*m*) of the frame is fixed first against that side of the block which faces the wide part of the trench (front in Fig. 3). Then the side boards are fastened to the back with four $2\frac{1}{2}$ in. nails driven through the holes of the guide hooks on the back (*k*). The next step is to tighten up the frame over the block. This is done by twisting the two- or three-ply hay wire catching projecting ends of the cross bars of the side boards. After this the bottom of the block is reinforced by driving three or four steel bars (*i*) of a suitable strength on the level with the bottom end of the back. The bars are long enough to go completely through the block and project at least two inches beyond its front side. The ends of the bars

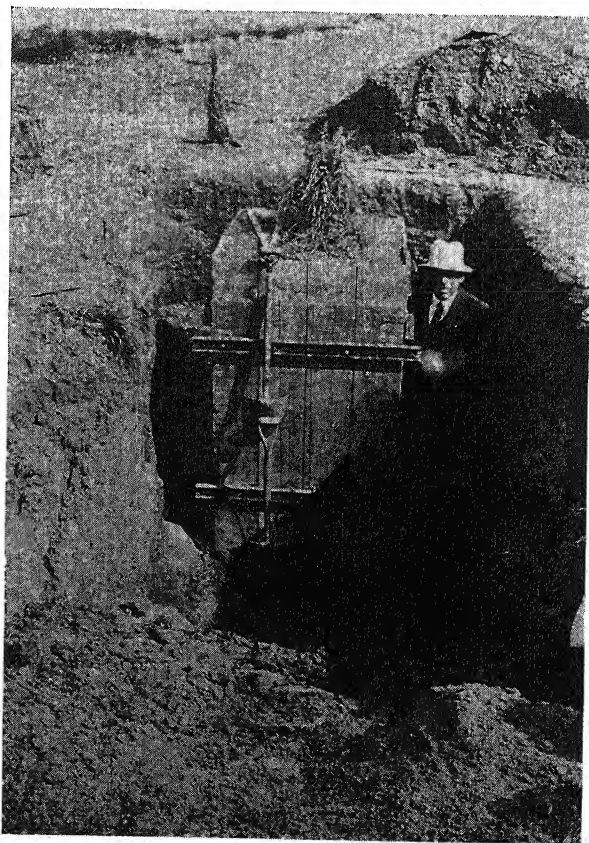


FIG. 3. A block of the same size as shown in Fig. 1, fully encased.

in front and at the back of the block are caught by wires and tightly fastened to the front lower stretcher (*g*) and the back lower cross bar (*h*). With very large blocks weighing from one to four tons, a piece of 2×8 in. plank of proper length is put under the front ends of the bottom bars, caught by chains equipped with hooks and turnbuckles, and tightly fastened to the upper cross bar of the back (*h*). The block thus reinforced is then carefully tipped over on its back in the hole.

8. *Elevating the Blocks*

In this position the block is ready to be raised to the surface. Four steel side-rods (*c*) are hooked on the special notches (*l*) in the cross bars of the back. The top ends of the side-rods then are connected in pairs by the lifting rods (*b*) to provide a suitable grip for the lifting hook of a block and tackle. To avoid possible damage to the block from the squeezing action of the side rods, wooden stretchers (*g*) are fitted in between each pair of the side rods to keep them apart at the distance of the width of the block. One or two strong wooden tripods, not less than 12 feet in height, and blocks and tackles of the required strength are used to raise the block to the surface.

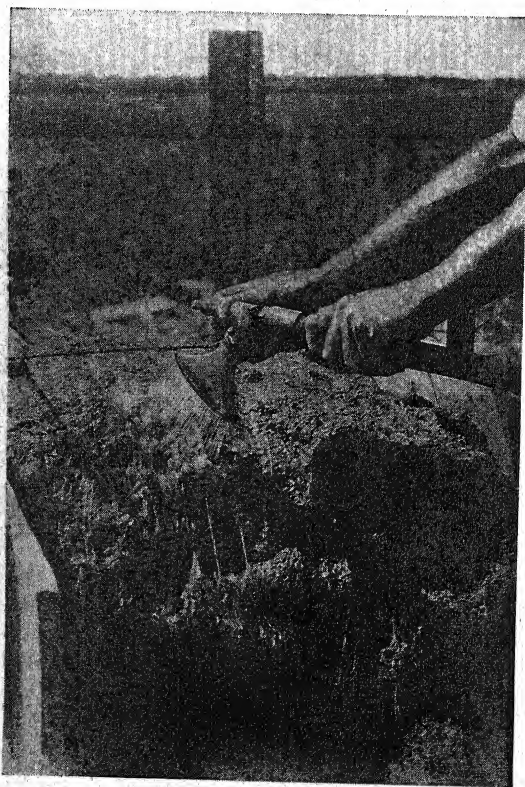


FIG. 4. *The adjustable nozzle for use in washing out the roots.*

9. Washing

In the past the main reason why the root systems were always so seriously damaged in the extricating process was that the force of the water used to dislodge the soil particles from the roots was too great for the tender root structures to withstand without breaking. This was also particularly true of methods used to remove the mass of the soil in a dry condition. It has been found, however, that the most delicate root structures can endure a surprisingly large amount of water action if the latter is applied properly. For this reason, in the soil-block washing method, the process of liberating the root material from the earth is done exclusively by water from a nozzle specially constructed for this purpose. This is shown in Fig. 4.

No root is touched by the hands or any other hard object until the last particle of soil is removed from the root system. To make the soil more susceptible to the action of water prior to washing, the block is thoroughly soaked in a large tank filled with water. Fig. 5 presents a general view of

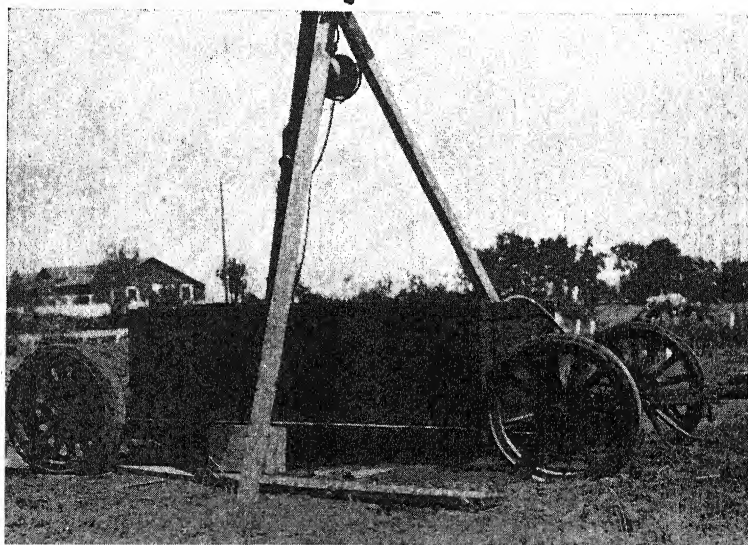


FIG. 5. A soaking tank $2\frac{1}{2} \times 4 \times 8$ feet.

the equipment. The tank is water-tight and constructed in such a manner that any one of its four sides can be easily removed and replaced when necessary. This is essential in handling the heavy blocks in order to obviate the necessity of lifting them high above the ground. For this purpose one side of the tank is removed and the latter is pushed under the block, which is suspended about 20 inches above the ground. As soon as the block is placed on the bottom of the tank, the side is replaced and the tank filled with water. The soaking lasts for several hours, larger blocks receiving more time than smaller ones. The washing is done with the greatest possible care by an experienced person. The stream of water is such as to be sufficiently

gentle for the minute root structures and yet strong enough to loosen and carry away the soil particles. A fan-shaped nozzle with small holes breaking the water stream from an ordinary one-inch garden hose into numerous fine jets seems to comply with both requirements exceptionally well. The strength and volume of the water stream is controlled by a hand operated valve conveniently located near the nozzle (see Fig. 4).

To determine such matters as (a) the greatest depth of penetration, (b) the lateral spread of the main roots, (c) the greatest depth at which side branches of the first order appear on the main roots, (d) the depth at which these produce branches of the second order, etc., the washing is started at the bottom of the block and proceeds upwards until the root system is liberated from the last speck of soil (see Fig. 6).

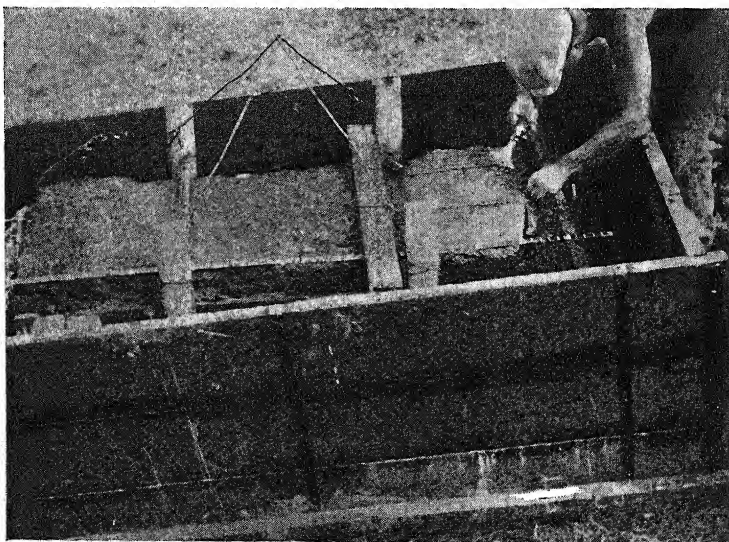


FIG. 6. *Method of washing soil blocks. Work begins at the bottom of the block so that the depth of penetration and lateral spread of the main roots and side branches may be precisely determined.*

10. Field Records

A report is kept for each block. This consists of two sheets. At the top of the first sheet the name of a plant or plants (in competition) studied is given as a general title. Then records concerning the dates of seeding, emergence and excavation are entered. In addition brief notes on the soil profile, moisture conditions at different depths and the most characteristic features of the root systems are included. On graph paper a pencil chart of the root system is made to scale. On this chart the exact position, depth of penetration, and lateral spread of each main root are indicated not only by location on the chart, but more precisely by numbers inserted in squares at the bottom of the sheet.

11. Preserving the Root System in Fresh Condition for Detailed Analysis

The charts mentioned in the preceding paragraph are very accurate. Each main root is carefully charted from the moment when its tip first appears at the bottom of a block until it joins the underground stem. Nevertheless, there are in each root system many very important details that cannot be recorded at the time of washing. These, however, can be clearly observed and accurately studied if the root system is placed in clear water in a suitable tank. Since time is not available in summer for the analysis of each root system as soon as it is washed, the root material is preserved in a fresh condition until winter for detailed analysis. The material is preserved in a 3 to 4% solution of formaldehyde. The younger plants are kept in glass jars and the large root systems in tanks, each labelled to correspond with the proper field report. With this simple treatment the root systems stored for several months look as natural and fresh as on the day of excavation.

12. Analysis

The analysis is done in special steel or wooden tanks. These are of such size as to receive an entire plant with both top growth and root system spread into their normal positions. In practice, tanks from 8 to 12 feet long, 4 feet wide and 8 inches deep, have proved to be satisfactory for most of the field crops and weeds. The tanks are painted black inside to give the greatest possible contrast between the background and white, or nearly white, root material. At one end each tank has a drain. At the left hand side of the bottom a white scale with one-inch and five-inch divisions is made to indicate the height of the top growth and the depth of each main root. A zero mark corresponding to the ground level is inserted on the scale. From this point on the scale the divisions are numbered upwards and downwards. Another white scale at right angles to the first is made to indicate the lateral spread

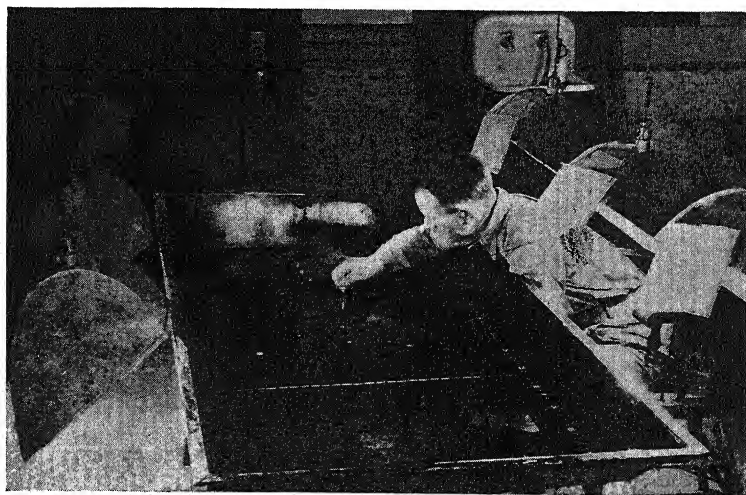


FIG. 7. *The analysing tank and facilities for illumination.*

Characters studied	Five plants studied					Totals	Averages
	1	2	3	4	5		
<i>Branches, Third Order</i>							
Max. No. per lin. inch.
Min. No. per lin. inch
Longest branch, inch
No. branch, per plant
Length per plant, inch
<i>Crown Root System</i>							
No. of crown roots
Longest root, inch
Shortest root, inch
Max. penetration, inch
Max. spread, inch
Length, less br., inch
<i>Branches, First Order</i>							
Max. No. per lin. inch
Min. No. per lin. inch
Longest branch, inch
No. branch, per plant
Length per plant, inch
<i>Branches, Second Order</i>							
Max. No. per lin. inch
Min. No. per lin. inch
Longest branch, inch
No. branch, per plant
Length per plant, inch
<i>Branches, Third Order</i>							
Max. No. per lin. inch
Min. No. per lin. inch
Longest branch, inch
No. branch, per plant
Length per plant, inch
Total length of root system per plant, inch

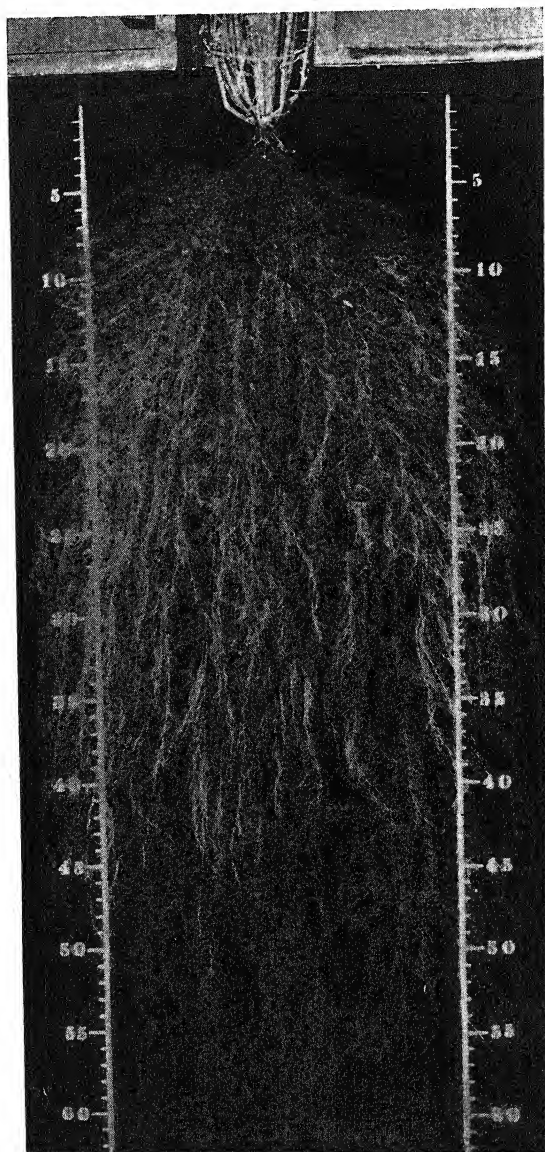


FIG. 8. The root system of a single wild oat plant, grown free from competition and excavated 80 days after emergence. Total length of roots 54 miles.

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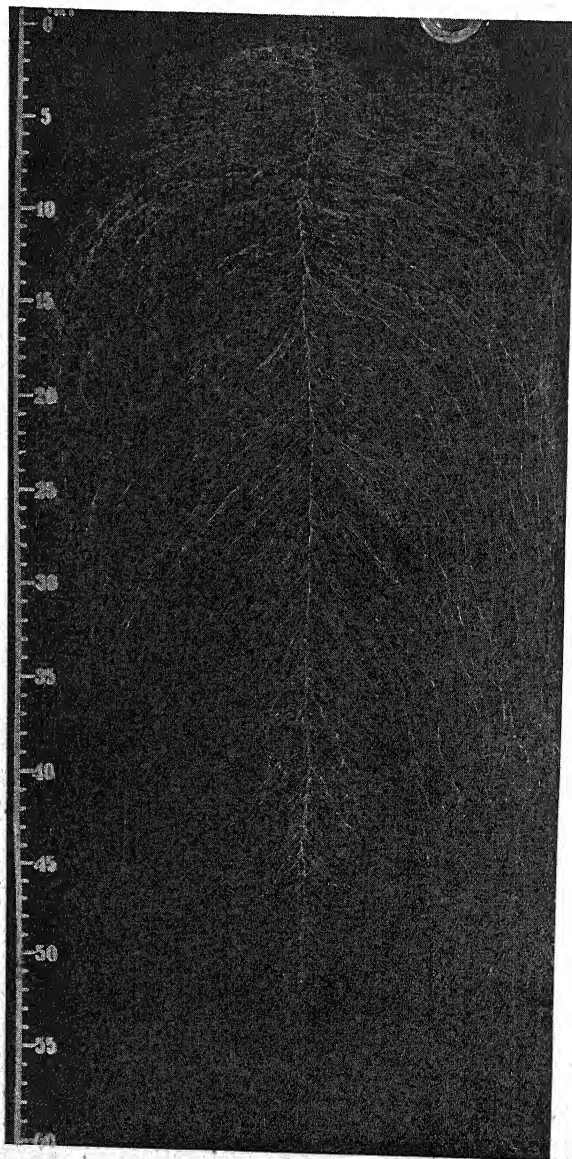


FIG. 9. One crown root from the root system shown in Fig. 8. Total length of this crown root, 4.05 miles.

TABLE I

DETAILED STUDY OF ONE CROWN ROOT SEPARATED FROM THE ROOT SYSTEM OF A SINGLE PLANT OF WILD OATS GROWN FREE FROM COMPETITION IN THE CENTRE OF AN AREA TEN FEET SQUARE

Characters studied	Data	Characters studied	Data
Length of the root	63 in.		
<i>Branches of first order</i>		<i>Branches of first order—Concluded</i>	
Longest branch	51 in.	Total number of branches of first order	676*
Frequency of branches at different depths:		Total length of branches of first order	19675**
0 to 5 in.	9		
6 to 10 in.	13	<i>Branches of the second order</i>	
11 to 15 in.	17	Longest branch	13 in.
16 to 20 in.	16	Average frequency per linear inch	13
21 to 25 in.	11	Greatest depth at which they occurred	48 in.
26 to 30 in.	11.6		
31 to 35 in.	10	Their total number	145500†
36 to 40 in.	12	Their total length	237000††
41 to 45 in.	10.4	Total number of first and second order branches	146176
46 to 50 in.	10.2	Their total length	256675 in.
51 to 55 in.	11		
56 to 60 in.	11		
61 to 65 in.	0		
Average length of branches of first order at different depths:			
0 to 5 in.	34 in.		
5 to 10 in.	41 in.		
11 to 15 in.	49 in.		
16 to 20 in.	46 in.		
21 to 25 in.	40 in.		
26 to 30 in.	36 in.		
31 to 35 in.	27 in.		
36 to 40 in.	18 in.		
41 to 45 in.	10 in.		
46 to 50 in.	4 in.		
51 to 55 in.	2 in.		
56 to 60 in.	0.75 in.		
61 to 65 in.	0.0 in.		

NOTE.—Branches of third and fourth orders were very numerous but not estimated.

*These were actually counted.

**The value represents the sum of results obtained by multiplying the actual number of root branches at each depth by their average lengths.

†This number was obtained on the basis of the length of branches of the first order actually bearing branches of the second order, multiplied by the average frequencies of the latter as determined from 250 random counts at each five-inch depth.

††The length of branches of the second order was arrived at in the following manner: starting from the surface downward, a hundred random counts and measurements were made at each consecutive five inches in depth, to determine the average frequency and length of the branches. The results thus obtained are summarized in the value indicated.

A study of the results presented in Table I shows that a multitude of root structures, many of which measure one twenty-fifth of a millimeter in diameter or less, may stretch over four feet in length through hard and frequently very coarse ground. By the use of the soil-block washing method in a joint study by the Dominion Forage Crops laboratory and the Weed Research Nursery

at Saskatoon, it was determined that the root systems of three-year-old single plants of slender wheat grass, brome grass and crested wheat grass measured 9.9, 65.2 and 315.4 miles respectively. Needless to say, the main roots in each case constitute only a negligible fraction of these enormously large quantities. A much greater portion is contributed by the branches of the first order and still more by those of the higher orders. This serves to indicate the importance of the youngest and finest root organs in the formation of the total absorption root surface, which determines the competitive efficiencies of plants. It is this most delicate root material that is most difficult to extricate from the ground and this complicates the problem of root study. However, the soil-block washing method seems to handle this task within the limits of practical needs, and from this point of view it has proved to be a valuable tool in determining the biological merits of economically important plants.

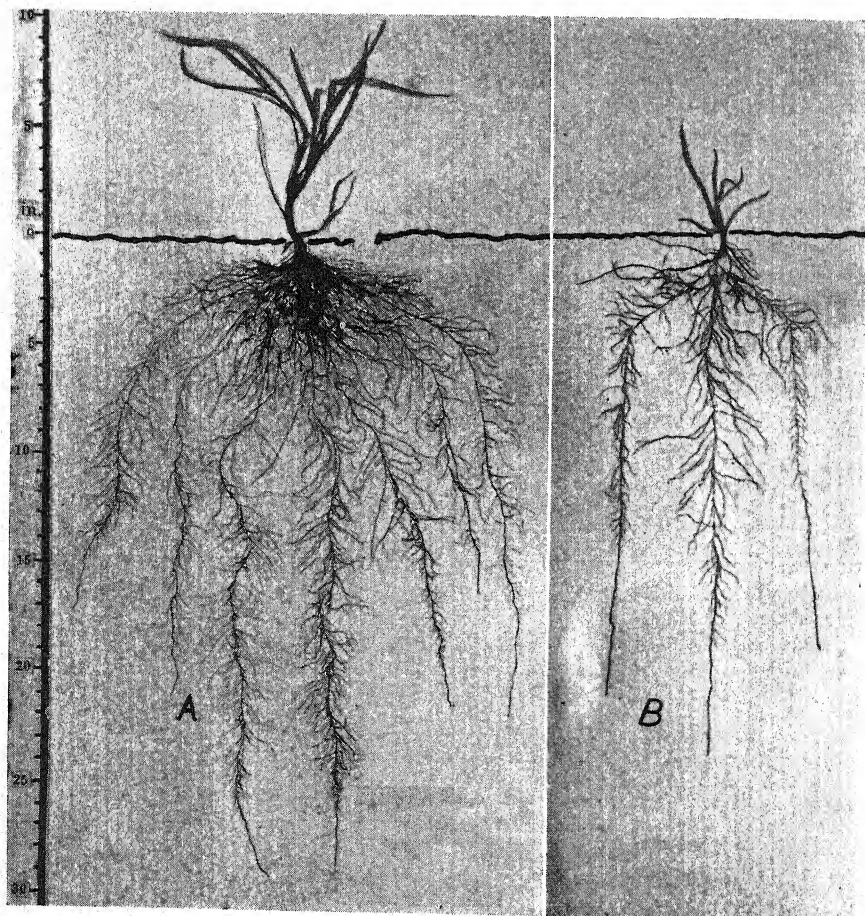


FIG. 10. Root systems of Hannchen barley (A) and wild oat (B) plants which grew side by side three inches apart. The illustration indicates size and distribution of the root systems. An indication of their interrelations in the soil would be obtained if B were superimposed on A, with the stems three inches apart. These root systems are so arranged on the original mount, from which the photographs were made in water. Twenty-two-day-old material.

13. Photographing the Root Systems after Analysis

It has already been mentioned that, for the analysis, each root system is properly spread in the water. Since the roots of most species are white or nearly white in color, it is easy to obtain sufficient contrast between them and the deep black bottom of the analyzing tank to photograph them right in the water, where they float freely and assume the most natural forms and positions (see Figs. 8, 9 and 10).

14. Bleaching Process for Dark Root Material

The root material of some species is so dark in color that there is not sufficient contrast between the roots and either a black or white background in the analyzing tank. It is therefore difficult to analyze such specimens, and not possible to photograph or mount them. Brome grass is typical of this class of material. The difficulty was overcome, however, by applying a bleaching process to reduce the dark pigment without impairing the natural strength of the roots. The process is as follows.

- (1) Soak roots in a mixture composed of 99 parts of the commercial preparation known as Javelle water, one part of 30% hydrogen peroxide and traces of chlorine water. Prepare sufficient of the mixture to cover the root material completely. The latter should remain in the mixture until the dark pigmentation disappears.

- (2) Wash the roots in clean soft water.

- (3) Bathe them for 10 minutes in thick suds of sodium oleate.

- (4) Wash very thoroughly in soft water, and drain.

- (5) Dip the roots in 95% alcohol for two minutes, drain and wash with soft water.

15. Mounting of Root Specimens

Mounting also is best done under water. In this case a suitable mounting background, prepared ahead of time, is slowly pushed under the root system. By careful manipulation of long needles, the main roots and their branches are moved into their proper places on the background and fixed by the Babbitt weights. As soon as this is done the drain cock is half opened and in a short time the root system is left in the natural shape on the mounting background. In mounting the root systems of unrelated species obtained from competition plots it is possible to dye these in different colors, spread them under water so as to represent their interrelation in the ground and finish the mounting as in the previous case.

This method of mounting requires a good deal of experience, but it certainly gives a splendid opportunity of demonstrating the nature, extent and distribution of root systems in a most satisfactory form. Employing this technique a very extensive collection of root systems of various cereal and forage crops and weed species has been established at the University of Saskatchewan. This collection may be regarded as the beginning of a root study museum.

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THE SYNTHETIC PRODUCTION OF OAT VARIETIES RESISTANT TO RACE 6 AND CERTAIN OTHER PHYSIOLOGIC RACES OF OAT STEM RUST¹

BY J. N. WELSH²

Abstract

At the present time, oat varieties that are classed as resistant to *Puccinia graminis Avenae* Erikss. & Henn. are only resistant to a certain number of the ten physiologic races. With the object of combining in a single variety resistance to as many races as possible, a cross was made between the varieties Hajira Strain and Joannette Strain. Hajira Strain is susceptible to Races 4, 6, 8, and 10, and Joannette Strain to Races 2, 6, 7, 8, and 9. The latter variety gives an indeterminate reaction to Races 5 and 10. Both parents are susceptible to Races 6 and 8.

From this cross 93 pure lines were obtained. Under greenhouse conditions, 71 were resistant at the seedling stage to Race 6 at 60° F. At 65°–70° F., approximately one-third of these were resistant to Race 6, one-third semi-resistant, and one-third susceptible. At more advanced stages of growth, namely, fifth-leaf, boot, and heading, representative lines from each of these classes were resistant to Race 6 at 60° F. At 65°–70° F. all showed regional resistance: at the fifth-leaf stage, the tip end of the uppermost leaf only was susceptible; at the boot stage, numerous pustules were present on the uppermost node and internode but the remaining parts were free from infection; at the heading stage, only one or two fairly large pustules occurred on the uppermost node or internode.

Six lines that were consistently resistant to Race 6 at 60° F. and 65°–70° F. were tested at the seedling stage at 60°, 65°–70° F., and 75°–80° F., to Races 1, 2, 3, 4, 5, 6, 7, 8, and 10. At the low and intermediate temperatures, these lines were resistant to the nine races. At the high temperature, they were susceptible to Race 6, gave an indeterminate reaction to Races 1, 4, and 5, and were resistant to all the other races.

Under field conditions, six lines classed as resistant at 65°–70° F., five classed as semi-resistant, and four as susceptible, were tested to Race 6. All these lines behaved similarly: infections of a semi-resistant type appeared on the uppermost internodes, while other parts of the plants were free from infection.

The standard varieties used as checks, namely, Hajira Strain, Joannette Strain, White Russian, and Victory, were susceptible to Race 6 in all the greenhouse experiments, and, with the exception of White Russian, in the field test. In the latter test, White Russian was semi-resistant.

Introduction

One of the many problems confronting the plant breeder is to combine in a single variety of oats resistance to all the physiologic races of *Puccinia graminis Avenae* Erikss. & Henn. Ten races (previously called forms) of oat stem rust are known at the present time and no variety is known to be resistant to all of them. The varieties classed as resistant to oat stem rust differ in their reactions to some of these races. Some are resistant to certain ones and susceptible to others; none are resistant to Race 6.

These varieties may be classified into three groups according to their seedling reaction to the several races. The first group is represented by Hajira Strain, which is resistant to Races 1, 2, 3, 5, and 7 and semi-resistant to Race 9;

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the second by White Russian, which is semi-resistant to Races 1, 2, 5, 8, 9, and 10; and the third, by Joannette Strain which is resistant to Races 1, 3, 4, and 10 and gives an indeterminate reaction to Race 5.

The infection types produced by the races to which Hajira Strain and White Russian are resistant are not affected by changes of temperature (See Table IV). The types of those to which Joannette Strain is resistant, on the other hand, are affected by such changes. Waterhouse (6) found that this variety was resistant to Race 1 at low temperatures, but completely susceptible to it at high temperatures. Gordon (1, 2) showed that Joannette Strain was susceptible, both at the seedling and mature stages, to Races 1, 3, 4, and 5 at 75.4° F., but was resistant to Races 1, 3, and 4, and produced an indeterminate reaction to Race 5 at 57.4° F. Newton and Johnson* found that Joannette Strain was resistant to Race 10 at 60° F., susceptible at 75°–80° F., and gave an indeterminate type of reaction at 65°–70° F.

Up to the present, not a great deal has been accomplished in the building up of resistance to the several physiologic races of oat stem rust. Smith (5) found that, in a Gopher × Rainbow cross, lines resistant or susceptible to Races 1, 2, 3, 5, and 7 reacted similarly to Race 8, but that lines segregating for resistance to this group of five races also segregated for resistance to Race 8. In a Hajira Strain × Joannette Strain cross (hereinafter referred to as Hajira and Joannette) Welsh (7) combined the resistance of these two varieties and obtained lines resistant to Races 1, 2, 3, 4, 5, and 7.

The present study deals with the reaction of pure line selections of this cross to nine of the ten races. Race 9 was not available for the study. These lines were inoculated in the greenhouse with the nine races at the seedling stage and with Race 6 at the more advanced stages of growth, at different temperatures. Some of the selections were also inoculated with Race 6 under field conditions. The results of these tests are presented in this paper.

Greenhouse Reactions to Race 6

Three F_3 lines of the Hajira × Joannette cross were inoculated with Race 6. A number of lines from this cross were previously shown (7) to be resistant to Race 4, a race to which Joannette is resistant, and to Races 1, 2, 3, 5, and 7, to which Hajira is resistant. To check further the resistance of these lines to Race 4, they were again inoculated with this race. Three lines that appeared particularly resistant to Race 4 in this test were inoculated with Race 6. One of the three lines was homozygous for resistance to this race, while the other two segregated. All of the resistant seedlings were transplanted and grown to maturity in the greenhouse. The progeny of these were then inoculated with Race 6 and again the most resistant seedlings were selected and transplanted. This procedure was continued until the plants had reached the sixth generation, by which time 93 pure lines were obtained.

* Unpublished data, Dominion Rust Research Laboratory, Winnipeg, Canada.

It was observed throughout these infection tests that a number of the seedlings gave an indeterminate type of reaction. It was also noted that the reactions, in general, were heavier when the temperature in the greenhouse was comparatively high.

These 93 lines were inoculated with Race 6 in January, during which month there was very little sunshine. The greenhouse was kept at 60°–65° F., owing to the presence of another experiment requiring that temperature. Of the 93 lines inoculated, 71 were resistant, 6 semi-resistant, 4 susceptible, and 12 gave an indeterminate type of reaction. In February, and again in

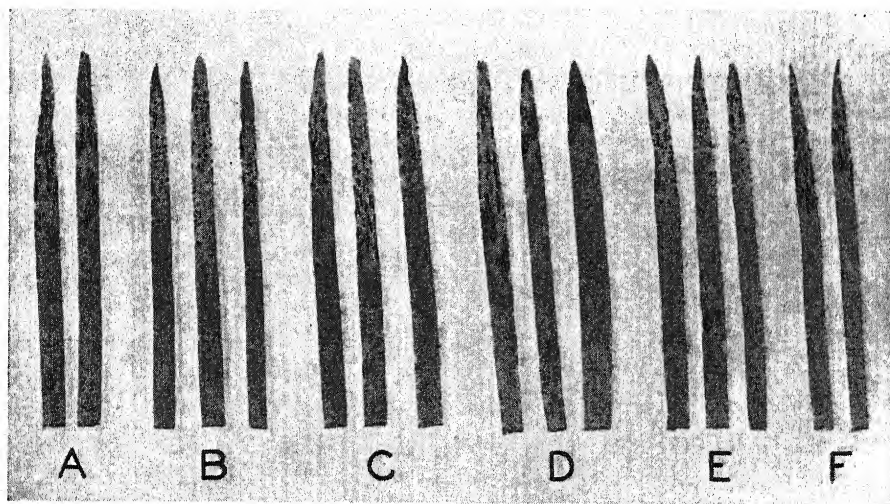


FIG. 1. Seedling reaction classes, at 65°–70° F., on lines of a Hajira × Joannette cross, inoculated with Race 6. A and F,—(Hajira and Joannette respectively), susceptible; B,—resistant; C,—semi-resistant; D,—susceptible; E,—indeterminate.

March, the 71 resistant lines were further tested to Race 6. There was practically continuous sunshine during the day throughout these two months and the temperature in the greenhouse was approximately 65°–70° F., although sometimes higher. Under these conditions the infections were heavier and more of the lines gave the indeterminate reaction than in the January test. The lines were classified according to their reactions into four groups: resistant, semi-resistant, indeterminate, and susceptible. The reaction classes observed during the two latter tests are shown in Fig. 1. As the lines reacted differently at the low and intermediate temperatures, experiments were planned to test the reaction of the lines under conditions of controlled temperature.

Effect of Temperature on the Reactions of Lines to Race 6 at Different Stages of Growth

In order to determine more definitely the effect of temperature on the reaction of the lines to Race 6, certain lines were tested at different temperatures at the seedling and more advanced stages of growth, namely, fifth-leaf, boot and heading.

Reactions at Seedling Stage

Twenty-four lines (eight from each of the lines formerly classified at 65°–70° F. as resistant, semi-resistant, and susceptible) were tested at 60° F. and 65°–70° F. Six of the eight resistant lines were tested at 75°–80° F. The standard varieties used as checks in this and subsequent tests were Hajira, Joannette, White Russian, and Victory.

At 60° F., all of the 24 lines were resistant. At 65°–70° F., the eight lines classed as resistant remained resistant and those of the semi-resistant and

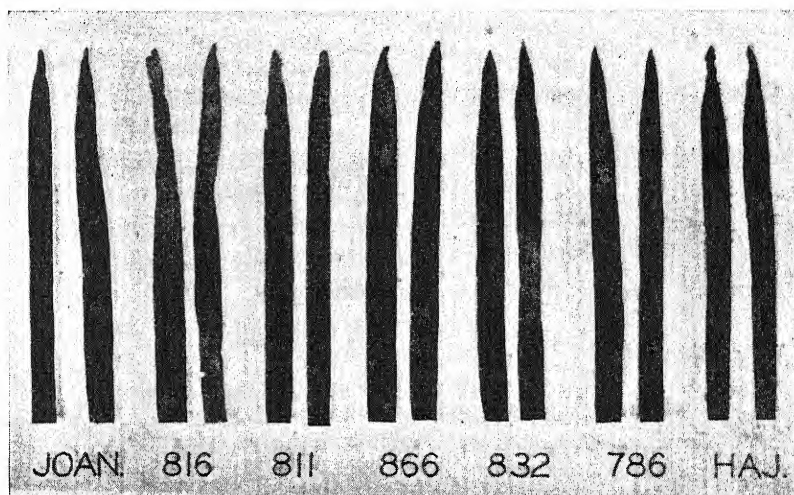


FIG. 2. Seedling reactions, at 60° F., of parents and lines of a Hajira \times Joannette cross to Race 6.

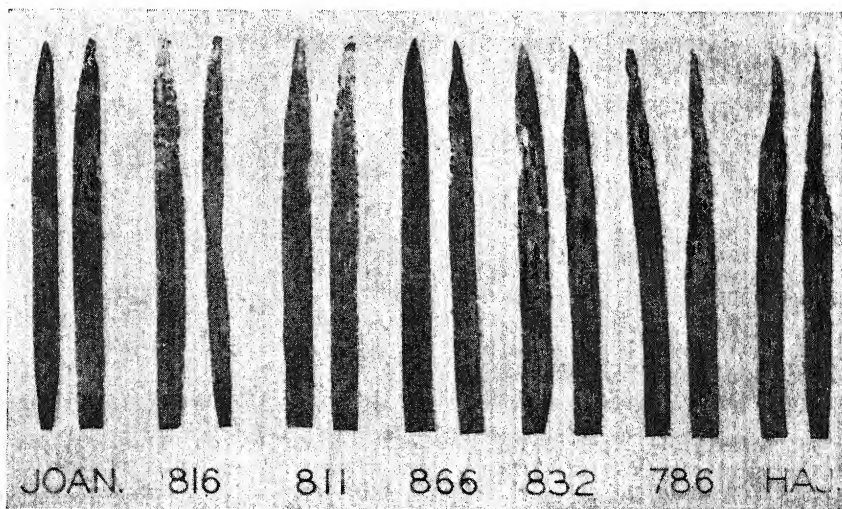


FIG. 3. Seedling reactions, at 65°–70° F., of parents and lines of a Hajira \times Joannette cross to Race 6.

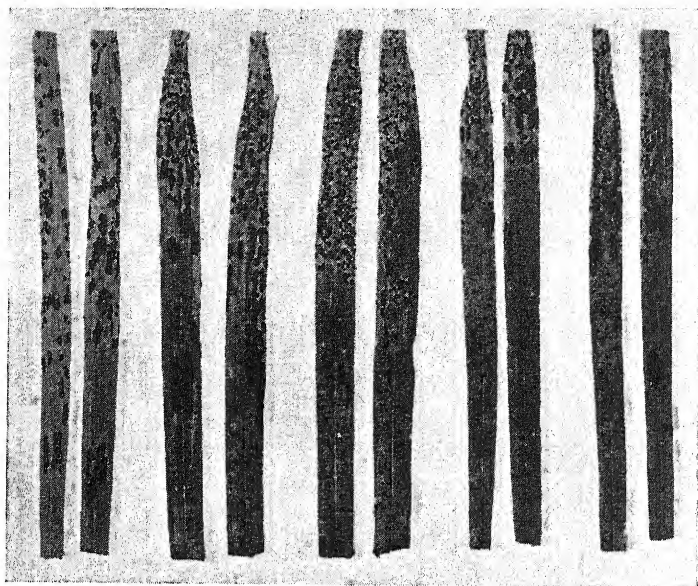


FIG. 4. Seedling reactions, at 75°–80° F., of the four standard varieties used as checks and one line of a Hajira \times Joannette cross, to Race 6. Left to right: Line 811, Victory, White Russian, Joannette, and Hajira.

susceptible classes again gave semi-resistant and susceptible reactions, respectively, thus confirming the former observations that at intermediate temperatures some lines become susceptible, while others remain resistant. At 75°–80° F., the six resistant lines tested were completely susceptible. The results show definitely that in the seedling stage the reactions of these lines to Race 6 are affected by temperature changes.

The reactions at the three temperatures are shown in Figs. 2, 3, and 4. Figs. 2 and 3 show the reactions at 60° F. and 65°–70° F., respectively, of two of the resistant lines (Nos. 816 and 811), one of the semi-resistant (No. 866), and two of the susceptible ones (Nos. 832 and 786), together with the reactions of the two parents. It will be observed that lines 832 and 786 gave a resistant reaction at the low temperature and a susceptible one at the higher temperature, while the reactions of the other lines showed little or no change. Fig. 4 shows the reaction at 75°–80° F. of one of the lines (No. 811) that was resistant at the two lower temperatures and the reaction of the four standard varieties.

Reactions at More Advanced Stages

As the reactions of the lines at the seedling stage were affected by temperature, an experiment was planned to determine whether the lines would show similar behavior at other stages of growth, namely, fifth-leaf, boot, and heading. Six lines, two from each of the groups previously classified at 65°–70° F. as resistant, semi-resistant, and susceptible, and the four standard varieties were inoculated with Race 6 in the above-mentioned stages. The

tests were conducted in duplicate at two temperatures 60° and 65°–70° F. Owing to the extremely cold weather that prevailed at the time the tests were conducted, temperatures higher than 70° were not obtainable. The lower temperature varied relatively little, but the higher temperature frequently dropped as low as 60° F. at night.

At 60° F., the six lines just referred to were resistant at all stages. At 65°–70° F., however, the reactions differed somewhat at the different stages, but all the lines reacted similarly at a given stage. At the fifth-leaf stage, all culms and leaves, with the exception of the uppermost leaf, were resistant. A portion of this leaf, from one to three inches back from the tip, was susceptible. At the boot stage, the leaves were resistant, whereas the culms gave a regional type of resistance in which the infections occurred only on the upper node and internode of each plant. At the heading stage, the leaves and culms were quite resistant, although generally one or two fairly large pustules could be found on the uppermost internodes. The standard varieties were susceptible at all stages at both temperatures. Temperature, therefore, affects the reaction of the lines at the more advanced stages of growth as well as in the seedling stage.

Field Reactions to Race 6

A field experiment was conducted in 1936 with six of the resistant, five of the semi-resistant, and four of the susceptible lines (so classed at 65°–70° F.)

TABLE I

FIELD AND GREENHOUSE REACTIONS OF STANDARD VARIETIES AND LINES OF A
HAJIRA × JOANETTE CROSS INOCULATED WITH RACE 6

Hybrid lines and varieties	Seedling reaction (65°–70° F.)	Field reaction			
		Range of pustule types	Rust percentages		
			Replicate 1	Replicate 2	Average
792	SR	3 – 3+	25	15	20
791	SR	3 – 3+	30	20	25
807	S	3 – 3+	40	20	30
811	R	3 – 3+	20	15	18
790	R	3 – 3+	15	30	23
806	S	3 – 3+	20	40	30
835	S	3 – 3+	25	15	20
863	SR	3 – 3+	15	25	20
814	SR	3 – 3+	15	30	23
839	S	3 – 3+	30	40	35
780	R	3 – 3+	15	25	20
846	R	3 – 3+	25	25	25
793	R	3 – 3+	35	35	35
862	SR	3 – 3+	30	30	30
794	R	3 – 3+	30	35	33
Hajira	S	4	40	35	38
Joanette	S	4	55	55	55
White Russian	S	2 – 3	25	30	28
Victory	S	4	35	55	45

Greenhouse reaction (seedling): R=resistant; SR=semi-resistant; S=susceptible.
Field reaction (Pustule type): 2=resistant; 3=semi-resistant; 4=susceptible.

to study their resistance to Race 6 under field conditions and to determine the relation between the field and the seedling reactions. The four standard varieties were used as checks. The seed was planted in single rod rows, in duplicate. Two guard rows of Victory were planted around the experimental plot. Inoculum of Race 6 was provided by transplanting heavily infected plants between the guard rows and also by injecting inoculum into the tissues of the plants in the guard rows on two occasions. When the plants were mature, rust percentage and pustule type were recorded. The data are summarized in Table I.

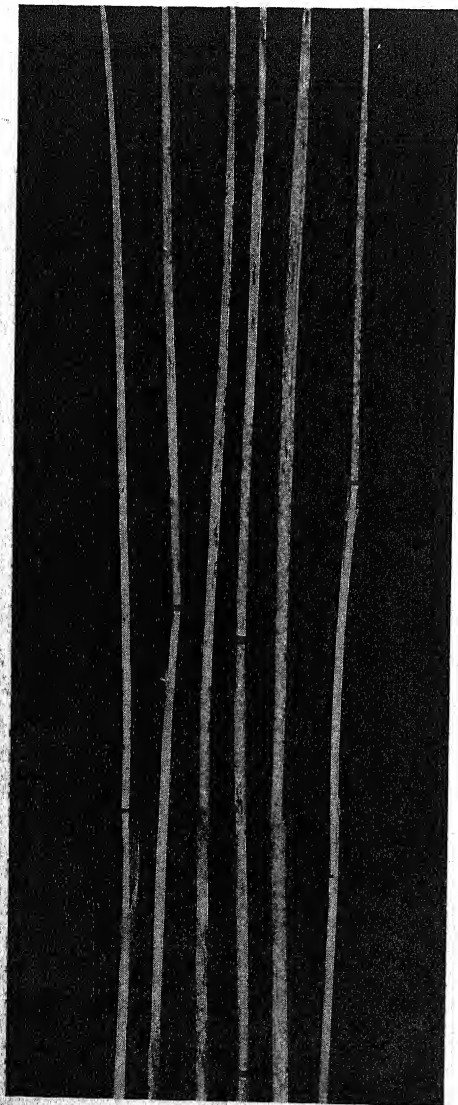


FIG. 5. Field reactions to Race 6 of the four standard varieties used as checks and two lines of a Hajira \times Joannette cross. Left to right: Line 811, Line 793, Hajira, Joannette, Victory and White Russian.

The lines gave a regional type of reaction, in which the infections occurred only on the uppermost internodes, a reaction similar to that produced in the greenhouse by the lines in the boot stage. The standard varieties, on the other hand, were rusted throughout the entire length of the culm. The rust percentages on all plants were based on comparable areas, namely, on the most heavily rusted six inches, and therefore do not represent the amount of rust present on the whole plant. On this account only slight differences in reaction between the lines and varieties appear in Table I. The average infection on the lines ranged from 18 to 35% and that on the standard varieties from 28 to 55%. The pustule types produced on the lines ranged from 3 to 3+, while those on the standard varieties, with the exception of White Russian, which gave a 2 to 3, were of the 4 type. White Russian had only 28% infection and produced a 2 to 3 type of pustule. This reaction is difficult to explain, as this variety is quite susceptible to Race 6 in the greenhouse, at all stages of growth. The reactions of two of the lines and the four standard varieties are shown in Fig. 5.

The data were analyzed statistically to determine whether the differences in reaction (i) between the lines and varieties, and (ii) within or between the resistant, semi-resistant, and susceptible classes, were significant. The results of the analysis are given in Table II.

TABLE II

ANALYSIS OF RUST PERCENTAGES ON STANDARD VARIETIES AND LINES OF A HAJIRA \times JOANETTE CROSS INOCULATED WITH RACE 6 UNDER FIELD CONDITIONS

Variance due to	Sums of squares	D.F.	Variance	F	5% point
Within resistant lines	485.4	5	97.1	1.57	2.77
Within semi-resistant lines	140.0	4	35.1	—	—
Within susceptible lines	237.5	3	79.2	1.29	3.16
Between groups of lines	123.8	2	61.4	—	—
Standard varieties	812.5	3	270.8	4.40	3.16
Lines vs. varieties	1533.7	1	1533.7	24.90	4.41
Replicates	65.8	1	65.8	1.07	4.41
Error	1109.2	18	61.6		
Total	4507.9	37			

The data in Table II show that no significant differences in reaction were obtained between lines within the resistant, semi-resistant or susceptible class, or between these three classes. It is evident, then, that as far as field resistance is concerned, all these lines react in a similar manner, regardless of their classification as resistant, semi-resistant, or susceptible at 65°–70° F. in the greenhouse. As the lines were all resistant at 60° F. their resistance at this temperature, therefore, is a criterion of their resistance under field conditions. To show further the lack of agreement between the greenhouse and the field reactions the data were arranged in a 2 \times 2 table. The field rust percentages were classified on a resistant, semi-resistant, and susceptible basis to correspond with the greenhouse classifications. The class range for each group was 5%. The data are given in Table III.

The data in this table show that there is no definite relation between the field reaction and the greenhouse reaction at 65°–70° F. Significant differences were obtained, however, within standard varieties, and between the varieties

TABLE III

RELATION BETWEEN THE RUST REACTION TO RACE 6 OF THE LINES OF A HAJIRA \times JOANETTE CROSS IN THE GREENHOUSE AT 65°–70° F. AND UNDER FIELD CONDITIONS

		Greenhouse		
		R	SR : S	
Field	R	3	4	7
	SR : S	3	5	8
		6	9	15

and lines. The fact that White Russian was more resistant than the other standard varieties accounts for the differences in reaction between the varieties. As the reaction of the lines and varieties differed significantly, it can be concluded that the lines are more resistant than the standard varieties.

Greenhouse Reactions to Other Physiologic Races at Different Temperatures

Six of the lines that were consistently resistant to Race 6 at the low and intermediate temperatures were inoculated at the seedling stage with other races at ordinary greenhouse temperatures (65°–70° F.) on three different occasions. As a further check on the resistance of these lines to Race 6, this race also was included in these tests. In the first two tests the lines were inoculated with Races 1, 2, 3, 4, 5, 6, 7, and 8, and proved resistant to all these races in both tests. In the third test Race 10 was included. The lines were resistant to this race also.

As the lines were resistant to Race 6 at the low and intermediate temperature, but susceptible to it at the high temperature, an experiment was planned to determine the reaction of the lines to the other races at 60°, 65°–70°, and 75°–80° F. Six of the most resistant lines, together with the four standard varieties, were inoculated with Races 1, 2, 3, 4, 5, 6, 7, 8, and 10 at these three temperatures. The six lines reacted similarly. The reactions of one of the lines, namely, No. 811, and of the four standard varieties are given in Table IV.

It will be seen in Table IV that this line (No. 811), representative of the other five lines, is not only resistant to the nine races at the low and intermediate temperatures but possesses, in general, a higher resistance than the standard varieties possess at these two temperatures to the races to which they are resistant. At 60° F., Line 811 gives a very resistant reaction to eight of the nine races and a resistant reaction to Race 6. At 65°–70° F., this line is resistant to Races 3 and 6, and very resistant to the other races. At 75°–80° F., it is resistant to Races 2, 3, 7, 8 and 10, gives an indeterminate type of reaction to Races 1, 4, and 5, and a susceptible reaction to Race 6.

Discussion of Results

The problem of developing oat varieties resistant to all physiologic races of *Puccinia graminis Avenae* is an important one as the resistant varieties which are at present grown commercially, or used for plant breeding purposes, possess resistance to only a certain number of the races. None of these varieties is resistant to Race 6. The resistance possessed by these varieties is effective only when the races present are those to which the variety or varieties in question are resistant. As races to which these varieties are susceptible sometimes appear in the field, it is desirable, therefore, to obtain oat varieties resistant to all races. An advance towards that objective is reported in this paper.

TABLE IV

THE REACTIONS OF THE STANDARD VARIETIES AND ONE LINE OF A HAJIRA X JOANETTE CROSS TO NINE PHYSIOLOGIC RACES OF STEM RUST AT LOW, INTERMEDIATE, AND HIGH TEMPERATURES—60° F., 65°-70° F., AND 75°-80° F., RESPECTIVELY

Varieties	Physiologic races																	
	1		2		3		4		5		6		7		8		10	
	Temp.		Temp.		Temp.		Temp.		Temp.		Temp.		Temp.		Temp.		Temp.	
	L	I	H	L	I	H	L	I	H	L	I	H	L	I	H	L	I	H
Line 811	VR	VR	I	VR	VR	R	VR	VR	I	VR	VR	I	VR	VR	R	VR	VR	R
Joanette	VR	I	S	S	S	S	R	I	S	I	S	S	S	S	S	S	R	I
Hajira	R	R	R	R	R	R	S	S	S	R	R	S	R	R	S	S	S	S
White Russian	SR	SR	SR	SR	S	S	S	S	S	SR	SR	S	S	S	SR	SR	SR	SR
Victory	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Temperature classification: L=low (60° F.); I=intermediate (65°-70° F.); H=high (75°-80° F.).

Reaction classification: VR=very resistant; SR=semi-resistant; I=indeterminate; S=susceptible.

Resistance to Races 1, 2, 3, 4, 5, 6, 7, 8, and 10 was obtained from a cross between the varieties Hajira and Joannette. Hajira, at all temperatures, is resistant to Races 1, 2, 3, 5, and 7, while Joannette, at low temperatures, only, is resistant to Races 1, 3, 4, and 10, and gives an indeterminate reaction to Race 5. Resistance to Races 6 and 8 was probably obtained through the medium of transgressive segregation, as neither parent is resistant to these two races.

The resistance of the lines at the seedling stage to Races, 1, 4, 5, and 6, and at the more advanced stages of growth to Race 6, was influenced somewhat by temperature. Furthermore, the reactions at the more advanced stages of growth in the greenhouse and at the mature stage in the field were regional in nature. At the seedling stage the lines were resistant to the nine races at the low and intermediate temperatures, but susceptible to Race 6, and gave an indeterminate reaction to Races 1, 4, and 5 at a high temperature. At the more advanced stages the lines were resistant in all stages at 60° F., but at 65°–70° F. they gave a regional type of resistance to Race 6 in which the infections occurred only on certain parts of the plant. At the fifth-leaf stage only the tip end of the uppermost leaf was susceptible, while at the boot, heading, and mature stage in the field only the uppermost nodes and internodes were infected.

As the lines were susceptible to Race 6 at the high temperature and gave an indeterminate reaction to Races 1, 4, and 5, three of the races to which Joannette gives an indeterminate reaction at the intermediate temperature, it is probable that this instability of reaction has been inherited from that parent. Further evidence in support of this hypothesis may be drawn from the fact that the reaction of Hajira, the other parent, to the races to which it is resistant, is not affected by changes of temperature.

The fact that, at the more advanced stages of growth, infections occurred only on certain parts of the plant indicates a regional type of resistance as suggested by Goulden *et al.* (3). These investigators observed that at the adult stage of certain wheat varieties, the region above the nodes and the culms between the uppermost leaf and the head rusted more heavily than other parts of the plants. Furthermore, Newton and Brown (4) have shown that the young rapidly growing parts of resistant varieties of wheat, oats, and barley, when inoculated by injection with a suspension of uredospores shortly before the plants come into head, are very susceptible, while the older, more mature parts are highly resistant. There appears to be considerable similarity between those results and the present findings.

That the infections only appeared at the tip end of the uppermost leaf at the fifth-leaf stage may be explained by assuming that only that portion of the leaf had protruded from the sheath at the time of inoculation, and being in a rapidly growing condition, became infected. On the other hand, this leaf was resistant at the boot stage. In fact, at the boot stage the only susceptible parts were the uppermost nodes and internodes. It is rather difficult to account for the appearance of pustules on these parts, as at the

time of inoculation they were enclosed within the sheath. Most likely the inoculum came into contact with them by entrance through the split in the sheath. These plants, being young and growing rapidly, were susceptible, and hence became infected.

Acknowledgments

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DETERMINATION OF THE DIASTATIC POWER OF MALT IN DEGREES LINTNER BY MEANS OF A FERRICYANIDE REAGENT¹

BY J. ANSEL ANDERSON² AND HENRY R. SALLANS³

Abstract

It is proposed that the Official Method, of the American Society of Brewing Chemists, for the determination of the diastatic power of malt be modified to permit the use of the Blish and Sandstedt ferricyanide method for determining the reducing power of the digested starch solution. The proposed method involves the use of half the quantity of infusion, rather than twice the quantity of starch, for making diastases of malts with Lintner values of over 135° L. Both changes increase the speed of determination without loss of precision or accuracy. For routine purposes additional speed can be obtained by omitting the dilution of the infusion and by requiring a blank correction for the reducing power of the starch only.

Experimental data show that under the conditions of the determination the ferricyanide method provides an accurate measure of the reducing power of the digested starch solution, and that the results obtained by the two methods, for 16 malts with diastatic powers covering the range from 72° to 185° L., agree to within 3%.

The Official Method of the American Society of Brewing Chemists for the determination of the diastatic power of malt (1, pp. 16-18), which has also been tentatively adopted by the Association of Official Agricultural Chemists (2, pp. 158-160) and which is being studied by the American Association of Cereal Chemists, leaves much to be desired as a routine method. Its unsuitability is amply proved by data given by Coleman (5) which show that 17 laboratories obtained results varying between 103° and 158° L. for the same malt.

These differences between laboratories may result from variations in technique at many stages of the procedure, but undoubtedly are due largely to the use of a titration of boiling Fehling's solution for the determination of the reducing power of the digested starch solution. This titration is notoriously unreliable since it is subject to large personal errors which can be overcome only by close adherence to detailed specifications and by standardizing the Fehling's solution under conditions identical with those used in the determination. These points have hardly received sufficient emphasis in the Official Method.

A second fault which reduces the usefulness of the method is its slowness. This again can be attributed largely to the use of Fehling's solution. Four titrations are required for each determination and additional time is required for rinsing and filling the burettes with different solutions.

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The possibility of changing two other features of the Official Method which add to its slowness might also be considered. It appears that official recognition should be given to the general practice of correcting Lintner values for the reducing power of the starch solution but not for that of the malt infusion. The correction for the latter is small and varies within narrow limits and for all practical purposes can be disregarded. It also seems desirable to return to the older method of carrying out the diastasis with 2 or 1 ml. of undiluted infusion and 100 ml. of starch solution in a 200 ml. flask. The dilution of the extract can be omitted with very slight loss of precision, and the use of smaller volumes of starch and the same size of flask for malts above 135° L. is advantageous in a routine laboratory. The adoption of these procedures effects a very considerable saving in the time required both for the actual determinations and the washing of glassware.

If any great improvement is to be made in the method for determining the diastatic powers of American malts, it appears that it will be necessary to make a radical change in the method of determining the maltose. Such a change was recently proposed by Gore and Steele (6), who describe a procedure involving the use of a modification of the Blish and Sandstedt ferricyanide method (2, pp. 218–221; 3 or 4). Their specifications, however, are not considered entirely satisfactory because the directions for making the malt infusion and the diastasis differ considerably from those of the Official Method; the neutralization of the digested starch solution and the use of large volumes for reduction and titration are unnecessary and time-consuming; and because the derivation of the conversion factor involves certain theoretical considerations of which no account is taken in the Official Method.

In the present paper a procedure is outlined for determining the maltose by means of the original semi-micro ferricyanide method of Blish and Sandstedt. There can be no doubt that this method is suitable for routine work since, as applied to the determination of the diastatic power of flour, it has received wide trial and is now recommended for adoption as an official method both by the American Association of Cereal Chemists, and by the Association of Official Agricultural Chemists. The method as applied to the determination of the Lintner values for malts has been in use for some time in three Canadian laboratories. The reduction in the time required to determine the reducing powers of the digested starch solutions is estimated as 50%, and there is general agreement that the method is more convenient and precise than the titration of boiling Fehling's solution.

In order to demonstrate the soundness of the ferricyanide method it is necessary to show: that under the conditions of the determination, the relation between the actual reducing power of digested starch solutions of various concentrations and their ferricyanide equivalents can be represented by a straight line having a slope of 45°; and that the ratio of the results obtained by the Official Method and the corresponding ferricyanide equivalents, for a series of malts covering a wide range of diastatic powers, is relatively constant. Data on these points are given in the experimental section.

There remains the problem of determining the factor for converting ferricyanide equivalents to degrees Lintner. Only a tentative solution can be offered. The difficulty lies in the fact that no one laboratory can state with assurance that its values, as obtained by the Official Method, are correct (*vide* Coleman (5)). The problem can best be solved by a co-operative investigation made in a number of laboratories, and it is hoped that the method outlined in this paper may prove sufficiently attractive to merit such an investigation by one or all of the three associations of chemists previously mentioned.

The Method

Malt Infusion and Starch Solution

Prepare the malt infusion and the buffered starch solution according to the Official Method of the American Society of Brewing Chemists (1, pp. 16-18).

Diastasis

Pipette 100 ml. of buffered starch solution into a 200 ml. volumetric flask and place it in a constant temperature bath maintained at 20° C. ($\pm 0.1^\circ$). When the solution has reached a temperature of 20° C. add exactly 2 ml. of malt infusion with a precision grade pipette. Rotate the flask during the addition and immediately thereafter tilt the flask so that the solution runs up over the side of the neck against which the pipette was drained. Maintain the mixture at 20° C. Exactly 30 min. after starting to add the infusion add 10 ml. of 0.5 *N* sodium hydroxide solution from a fast flowing pipette. Mix, make up to the mark with distilled water and again mix thoroughly. If the malt has a diastatic power of over 135° L. make the diastasis by the same method but use 1 ml. instead of 2 ml. of infusion.

If the 2 ml. and 1 ml. pipettes are of precision grade and thoroughly clean, if the outside of the delivery tube is wiped with a clean cloth before the volume is adjusted, and if the pipette is drained against the wet side of the flask neck for exactly 15 sec. after free flow ceases, then no appreciable loss of precision results from the procedure outlined above.

Starch Blank

Prepare the starch blank by adding 10 ml. of 0.5 *N* sodium hydroxide solution to 100 ml. of buffered starch solution in a 200 ml. volumetric flask and make up to the mark with distilled water.

Determination of Reducing Power

Determine the reducing power of the digested starch solution and of the starch blank by means of the ferricyanide method of Blish and Sandstedt (2 pp. 218-221; 3 or 4), using 5 ml. of solution and 10 ml. of ferricyanide reagent.

The reductions may be made in 100 ml. wide-mouth hard glass Erlenmeyer flasks covered with small crucibles. Results obtained with this technique and with the original one agree to within 0.01 ml. The modification saves time by eliminating the transfer of the reduction mixture from test tube to flask and permits the determinations to be run in batches of four or

six, the first batch being titrated while the second is in the boiling water bath, and so on. This leads to a more continuous procedure and greater accuracy (Compare Putman, Blish and Sandstedt (9)).

Calculation

Subtract the number of millilitres of sodium thiosulphate required to titrate the digested starch reduction mixture from the number of millilitres required to titrate the starch blank reduction mixture. The resulting number is the ferricyanide equivalent of the diastatic power of the malt. To convert the results to degrees Lintner multiply the ferricyanide equivalent by 18 when 2 ml. of malt infusion was used and by 36 when 1 ml. was used.

For routine laboratories it may be more convenient to use 0.0278 *N* sodium thiosulphate solution for which the conversion factors are 10 and 20.

Standardization of Sodium Thiosulphate Solution

As Blish and Sandstedt (3) have pointed out, standardization of the sodium thiosulphate solution is unnecessary if it is made up according to their directions. However, if it is desirable to standardize it for special work, the use of potassium iodate, recommended by Hanes (7), is rapid and trustworthy.

Experimental

Relation Between Reducing Powers of Digested Starch Solutions and Corresponding Ferricyanide Equivalents

An infusion was prepared from a malt having a diastatic power of approximately 160° L., and after diluting 1 to 5, aliquots of 10 ml. were used to digest 100 ml. quantities of buffered starch solution. After adding 10 ml. of 0.5 *N* sodium hydroxide all the solutions were mixed without further dilution. A supply of starch-infusion blank solution was prepared in a similar manner. Various quantities of the digested starch solution were measured into tared 200 ml. volumetric flasks, weighed and then made up to 120 ml. with the starch-infusion blank solution. Finally, the flasks were made to the mark with distilled water and the reducing powers of the solutions were determined by the ferricyanide method.

TABLE I
RELATION BETWEEN REDUCING SUBSTANCES PRESENT AND FOUND IN DIGESTED STARCH SOLUTIONS

Weight of digested starch solution, gm.	Ferricyanide equivalent, ml.	Reducing power, °L.		Difference, °L.
		Present	Found	
104.45	7.59	136.6	136.6	0.0
97.38	7.14	127.4	128.5	-1.1
90.35	6.55	118.1	117.9	0.2
83.28	6.08	108.9	109.4	-0.5
76.30	5.52	99.7	99.4	0.3
69.31	5.06	90.5	91.1	-0.6
62.27	4.50	81.4	81.0	0.4
55.22	4.01	72.2	72.2	0.0
48.17	3.46	62.8	62.3	0.5
42.14	3.06	55.1	55.1	0.0

The relative quantities of reducing substances present in each solution, calculated from the weights of digested starch solution, and the ferricyanide equivalents found are reported in Table I. The data have been converted to degrees Lintner to show the range of Lintner values which the experiment covers. Fig. 1 shows the graph obtained by plotting calculated values against values found.

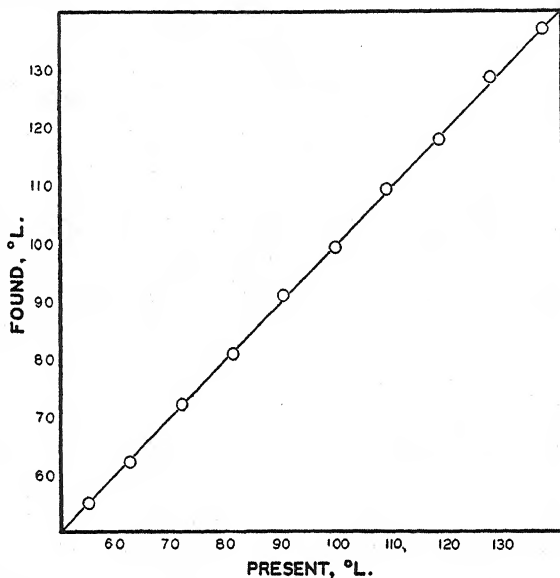


FIG. 1. *The relation between reducing substances present and found in digested starch solutions.*

It will be obvious that the experimental results are applicable to a double range of Lintner values, namely, from 55° to 136°, and from 110° to 272°, because the amounts of reducing substances produced during diastasis fall in the same range irrespective of whether 2 ml. or 1 ml. of infusion is used. The data show that under the conditions of the determinations of the diastatic powers of malts, the ferricyanide method provides an accurate measure of the reducing powers of the digested starch solutions.

Relation Between Results Obtained with Official and Ferricyanide Methods

The results obtained by following the directions for the titration, given in the Official Method, were not sufficiently precise, and it was therefore necessary to superimpose on them the more precise specifications of Lane and Eynon (8). The possible bias introduced by this change was overcome by standardizing the Fehling's solution by titrating it under exactly the same conditions with a solution containing 2 mg. of invert sugar per millilitre, and by adjusting the concentration of the Fehling's solution to correspond with a titre of 25.65 ml., the value given in Lane and Eynon's table for invert sugar (8). With the exception of this modification the directions given in the Official Method were followed exactly.

Eight malts were selected with diastatic powers between 70° and 140° L. Infusions were prepared from duplicate samples of each malt, and duplicate diastases and starch-infusion blanks were made using 2 ml. of each infusion. The reducing power of each digested starch solution and the corresponding blank correction were determined by both the Official and ferricyanide methods. A similar study was also made with eight malts with diastatic powers above 135° L. With these malts it seemed unwise to use the same digested starch solutions for both methods because the Official Method specifies that the diastasis shall be made in a 250 ml. flask with 10 ml. of diluted infusion, 200 ml. of buffered starch solution and 20 ml. of 0.5 *N* sodium hydroxide solution, whereas the ferricyanide method requires the use of a 200 ml. flask, 1 ml. of infusion, 100 ml. of buffered starch solution, and 10 ml. of 0.5 *N* sodium hydroxide solution. The former procedure is unsatisfactory for the latter method because it increases the concentration of reducing substances and thus lowers the upper limit which can be obtained with the ferricyanide reagent, and because it involves a change in the alkalinity of the solution which affects the relation between the ferricyanide equivalent and the actual reducing power of the solution. For these reasons separate diastases were made for each method in the second study.

The results of the two studies are reported in Table II. The data represent the means of determinations made on quadruplicate digested starch solutions.

TABLE II
COMPARISON OF RESULTS OBTAINED BY THE OFFICIAL AND FERRICYANIDE METHODS

Malt No.	Official method, °L.	Ferricyanide equivalent, ml. 0.05 <i>N</i>	Ratio, °L. Official to ferricyanide equivalent	Ferricyanide method, °L.*	Difference, °L.
1	72.4	3.88	18.66	69.8	2.6
2	79.7	4.34	18.36	78.2	1.5
3	88.7	4.85	18.29	87.3	1.4
4	98.9	5.42	18.25	97.5	1.4
5	104.3	5.73	18.20	103.1	1.2
6	118.0	6.60	17.88	118.8	-0.8
7	129.8	7.38	17.59	132.8	-3.0
8	137.4	7.72	17.79	139.0	-1.6
Mean	103.7	5.74	18.07	103.3	0.4
9	157.6	4.32	36.48	155.5	2.1
10	162.5	4.43	36.68	159.5	3.0
11	169.8	4.62	36.75	166.3	3.5
12	173.3	4.69	36.95	168.8	4.5
13	173.6	4.76	36.47	171.3	2.3
14	176.6	4.85	36.41	174.6	2.0
15	179.5	4.89	36.71	175.9	2.2
16	185.3	4.99	37.13	179.5	5.8
Mean	172.3	4.69	36.74	169.0	3.3

* Ferricyanide equivalents were converted to degrees Lintner by multiplying by factors of 18 and 36 in the upper and lower halves of the table, respectively.

Factors of 18 and 36 were selected for converting ferricyanide equivalents to degrees Lintner since the differences between these whole numbers and the ratios corresponding to 100° and 200° L. appeared to be insignificant.

It will be observed that the data in the upper half of the table show that the ratios between the results of the Official Method and the corresponding ferricyanide equivalents decrease fairly steadily with increasing diastatic power. This point is not so well illustrated by the second study in which the experimental errors were greater and the malts covered a narrower range of diastatic powers. It is worth noting, however, that in the second study the mean factor of 36.74 for a diastatic power of 172° L., which corresponds to a factor of 18.37 for a diastatic power of 86° L. in the lower range, agrees fairly well with the data reported in the upper half of the table.

In view of the data on the accuracy of the ferricyanide method reported in Table I, and for reasons given below, it seems fair to attribute the discrepancies between results obtained by the two methods almost wholly to the inaccuracy of the Official Method. When the ferricyanide equivalents are converted to degrees Lintner using a constant factor it turns out that, within each range, the Official Method overestimates the lower diastatic powers and underestimates the upper ones.

The quantity of maltose required to reduce a constant volume of Fehling's solution falls with increasing final volume of the reaction mixture, *i.e.*, with decreasing concentration of the unknown solution (Lane and Eynon (8)). When the titration of Fehling's solution was first adopted for the determination of Lintner values no correction was applied for this volume effect presumably because it was small. However, when the titration is used under the conditions outlined by the Official Method, this error becomes appreciable owing to the greater variation in the final volume of the reaction mixture caused by the method of determining the blank correction. In these circumstances it will be obvious that if a standard value is taken in the middle of the Lintner scale for each range, then the Official Method will overestimate the lower values and underestimate the upper ones, and as a net result the curvilinear nature of the relation expressed by Kjeldahl's law will be over-emphasized.

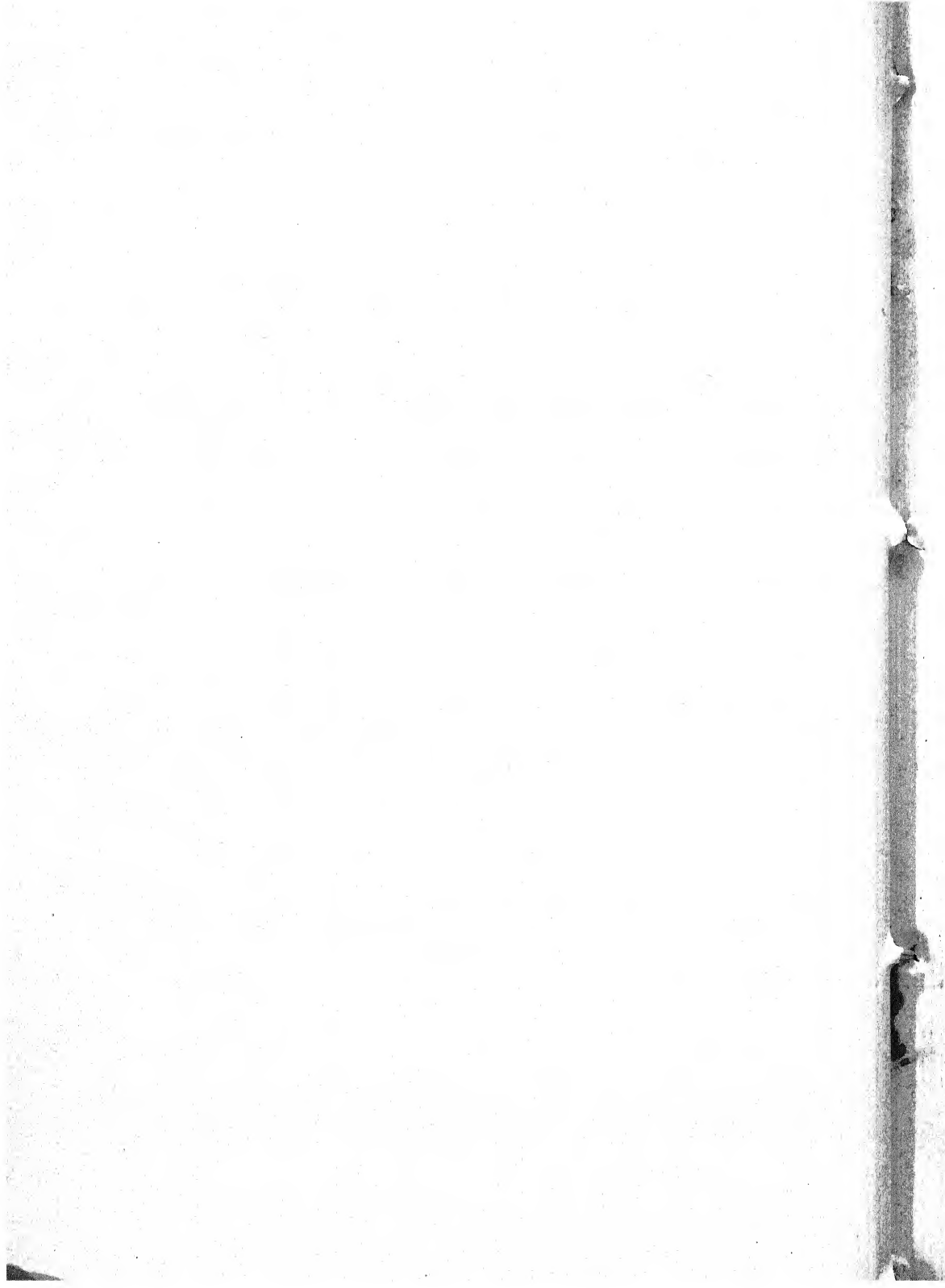
The Precision of the Proposed Method

The proposed method has been tested under a routine schedule, which required that the malts for several days' analyses be split into duplicate samples and arranged in random order. The analyst then made 12 single determinations, in random order, per day and washed all glassware used. Under these conditions the differences between duplicate samples are the result of all errors inherent in the method. In the National Research Laboratories, Ottawa, the standard error of the mean of duplicate determinations was found to be $\pm 0.94^\circ$ L. for 30 pairs of determinations involving the use of 2 ml. of infusion, and $\pm 1.06^\circ$ L. for 84 pairs involving the use of 1 ml. of infusion. In the malting laboratory at the University of Manitoba, Winnipeg,

under the same conditions, the standard error for 112 pairs of determinations was found to be $\pm 1.07^\circ \text{L}$. These data appear to represent a satisfactory level of precision for routine analysis.

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THE NITRATE REDUCTION TEST AND ITS SIGNIFICANCE IN THE DETECTION OF *BACILLUS LARVAE*¹

BY A. G. LOCHHEAD²

Abstract

Bacillus larvae differs from most nitrate-reducing species in its ability to accumulate nitrite in nutrient solutions containing but small amounts of nitrate (0.001%). Most nitrate-reducing organisms show no accumulation of nitrite at this concentration owing to assimilation of nitrate or disappearance of nitrite through reduction or assimilation. With many nitrate-reducing bacteria disappearance of nitrite keeps pace with nitrite formation only up to a certain concentration, varying with the organism, above which nitrite may accumulate.

The ability of *B. larvae* to accumulate nitrite in semi-solid carrot or turnip extract media with no added nitrate is of considerable aid in the cultural test for this organism. Of five other organisms concerned with brood disease or occurring as contaminants in comb, which were grown in association with *B. larvae*, none showed interference with accumulation of nitrite by the latter except *B. orpheus*. With this species a positive nitrite test was dependent on the relative development of the organisms, *B. larvae* exerting a certain antagonistic action. None of the eight species of bacteria tested prevented recognition of growth of *B. larvae* in the semi-solid medium.

Introduction

The application of the nitrite test has been found to be of considerable diagnostic value in the detection of *Bacillus larvae*, the organism causing American foulbrood of bees. As reported previously by the writer (4), this organism, grown in a suitable medium containing carrot extract, and without added nitrate, gives a positive reaction for nitrite. Under similar conditions all other spore-forming organisms so far tested, as well as many miscellaneous types that may occur in foulbrood diseases or as common contaminants, have not given a similar positive test.

Inclusion of the nitrite test in the routine cultural control for viable spores of *B. larvae* has confirmed its usefulness. Sturtevant (6) likewise found it a fairly delicate and reliable indicator of vegetative growth of this organism, while recently Hitchcock (3) reports successful application of the test. Even in the absence of visible growth, a positive nitrite reaction may be regarded as presumptive evidence of the presence of viable cells of *B. larvae*.

The experiments here described were undertaken to study more closely:—

- (i) The relation of *B. larvae* and other organisms to nitrate reduction and nitrite accumulation.
- (ii) The possible influence of contaminants in affecting the recognition of *B. larvae* and its characteristic nitrite reaction.

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Experimental

(1) Effect of Different Concentration of Nitrate on the Nitrite Reaction

A study was made of the effect of additions of potassium nitrate in varying concentration to media inoculated with *B. larvae* and a number of stock strains of organisms which showed nitrate reduction in standard broth containing 0.1% potassium nitrate. Using two basic solutions of yeast-peptone-carrot broth and beef-peptone broth, media were prepared containing 0.1%, 0.01% and 0.001% potassium nitrate and inoculated respectively with three strains of *B. larvae* and six other organisms known to be nitrate-reducing species. Yeast-peptone-carrot media were included in order to provide a series supporting good growth of *B. larvae*, an organism which does not develop in standard nutrient broth. This basic medium is prepared by adding to 1 litre water, 3 gm. yeast extract (Difco), 10 gm. peptone (Difco), 0.5 gm. di-potassium hydrogen phosphate (K_2HPO_4) and 200 ml. of clear carrot extract prepared by mincing 100 gm. carrots, grinding with 250 ml. distilled water and filtering (5).

The results of the test for nitrite, as well as for nitrate in cases where the former was negative, are shown in Table I. It will be seen that *B. larvae*, provided the medium is suitable for growth, gives a positive test for nitrite irrespective of the concentration of potassium nitrate used, showing a positive test in the yeast-peptone-carrot medium without added nitrate, due probably

TABLE I
NITRITE ACCUMULATION BY *B. larvae* AND VARIOUS NITRATE-REDUCING ORGANISMS IN DIFFERENT MEDIA

Organism	Yeast-peptone-carrot broth											
	+0.1% KNO ₃			+0.01% KNO ₃			+0.001% KNO ₃			No KNO ₃ added		
	Growth	NO ₂	NO ₃	Growth	NO ₂	NO ₃	Growth	NO ₂	NO ₃	Growth	NO ₂	NO ₃
<i>B. larvae</i> (Str. 1)	+	+		+	+		+	+		+	+	
<i>B. larvae</i> (Str. 2)	+	+		+	+		+	+		+	+	
<i>B. larvae</i> (Str. 3)	+	+		+	+		+	+		+	+	
<i>B. cereus</i> (135)	+	+		+	-	-	+	-	-	+	-	-
<i>Bacillus</i> sp. (101)	+	+		+	-	-	+	-	-	+	-	-
<i>Esch. coli</i> (117)	+	+		+	-	-	+	-	-	+	-	-
<i>Salmonella</i> sp. (136)	+	+		+	-	-	+	-	-	+	-	-
<i>Ebert. typhi</i> (116)	+	+		+	-	-	+	-	-	+	-	-
<i>Micrococcus</i> sp. (169)	+	+		+	+		+	-	-	+	-	-
Culture	Beef-peptone broth											
	+0.1% KNO ₃			+0.01% KNO ₃			+0.001% KNO ₃			No KNO ₃ added		
	Growth	NO ₂	NO ₃	Growth	NO ₂	NO ₃	Growth	NO ₂	NO ₃	Growth	NO ₂	NO ₃
<i>B. larvae</i> (Str. 1)	-	-	+	-	-	+	-	-	sl.	-	-	-
<i>B. larvae</i> (Str. 2)	-	-	+	-	-	+	-	-	sl.	-	-	-
<i>B. larvae</i> (Str. 3)	-	-	+	-	-	+	-	-	sl.	-	-	-
<i>B. cereus</i> (135)	+	+		+	-	-	+	-	-	+	-	-
<i>Bacillus</i> sp. (101)	+	+		+	+	-	+	-	-	+	-	-
<i>Esch. coli</i> (117)	+	+		+	-	-	+	-	-	+	-	-
<i>Salmonella</i> sp. (136)	+	+		+	sl.	+	+	-	-	+	-	-
<i>Ebert. typhi</i> (116)	+	+		+	+	-	+	-	-	+	-	-
<i>Micrococcus</i> sp. (169)	+	+		+	-	-	+	-	-	+	-	-

to the presence of traces of nitrate derived from the carrot extract. On the other hand, the other organisms tested, though giving a nitrite test with media containing 0.1% potassium nitrate, failed for the most part with 0.01%, and gave entirely negative tests with 0.001% potassium nitrate. The negative tests for nitrate where no nitrite was found indicate that with the lower concentrations of nitrate, there has been either assimilation of nitrate or disappearance of nitrite, through further reduction or by assimilation. In the case of *B. larvae* neither reduction of nitrites nor assimilation of nitrite or nitrate is apparent.

(2) *The Nitrite Test as Affected by Varying Concentrations of Nitrate and Nitrite in the Medium*

To examine further the behavior of different organisms to the nitrite test, two series of solutions were prepared from standard nutrient broth (Difco), one series with seven concentrations of potassium nitrate, from 0.1% to 0.001%, the other with equivalent amounts of potassium nitrite, from 0.0842% to 0.00084%. Tubes in both series were inoculated uniformly with a loopful of suspension of the organism tested. In all, 42 organisms were studied, 40 of which were nitrate-reducing in standard 0.1% potassium nitrate solution, with two non-reducing species as controls. The organisms consisted of various stock strains together with miscellaneous types freshly isolated from soil, air, milk, etc., some of which were not specifically identified. All cultures were incubated at 37° C. and tested for nitrite after one, two and seven days by the α -naphthylamine-sulphanilic acid test.

Of the 40 nitrate-reducing types studied only two gave positive nitrite tests with all concentrations of potassium nitrate. The remaining 38 species gave negative results in the lower concentrations to an extent which varied with the species. In Table II are shown results from typical members of this group (Group A) as well as from the two former (Group B) and the two non-reducing controls (Group C).

A comparison of the results for nitrate broth with those for nitrite broth of equivalent concentration shows general agreement in the limiting concentrations giving positive nitrite tests for the organisms of Group A. The results suggest that the negative nitrite tests with the lower concentrations of nitrate are due to further reduction or assimilation of nitrite, preventing any accumulation of the latter. This is confirmed by the frequent disappearance of nitrite, on prolonged incubation, from cultures which gave positive tests after one day. With some organisms nitrites were absent more promptly in the nitrite series than in cultures with equivalent concentrations of nitrate. This is understandable in view of a probable lag in the latter series as compared with that where nitrite is present originally.

Disappearance of nitrites apparently keeps pace with formation of nitrites only up to a certain concentration, varying with the organism, and above which nitrite may accumulate. The results indicate the importance of a sufficiently high concentration of nitrate in media used for the determination

TABLE II
EFFECT OF VARYING CONCENTRATIONS OF NITRATE AND NITRITE ON THE NITRITE TEST

Test organism		Nitrate broth—per cent KNO ₃ —(NO ₂ test after 1, 2, 7 days)																				
		0.1%			0.05%			0.02%			0.01%			0.005%			0.002%			0.001%		
		1	2	7	1	2	7	1	2	7	1	2	7	1	2	7	1	2	7	1	2	7
Group*	Name	Cult No.																				
A	<i>B. subtilis</i>	246																				
A	<i>B. cereus</i>	135																				
A	<i>B. mycoides</i>	381																				
A	<i>B. albolactis</i>	213																				
A	<i>B. pseudotetanicus</i>	154																				
A	<i>B. orpheus</i>	226																				
A	<i>Esch. coli</i>	117																				
A	<i>Aerob. cloacae</i>	133																				
A	<i>Salmonella</i> sp.	136																				
A	<i>Ed. typhi</i>	251																				
A	<i>Staph. aureus</i>	292																				
B	<i>Micrococcus</i> sp.	329																				
B	<i>Flavobacterium</i> sp.	170																				
C	<i>B. vulgatus</i>	389																				
C	<i>B. alvei</i>	127																				
D	Control—not inoc.	—																				

*Group A—Nitrate reduced to nitrite, partial reduction or assimilation of nitrite.

Group B—Nitrate reduced to nitrite, no reduction or assimilation of nitrite.

Group C—No reduction of nitrate or nitrite.

TABLE II—*Concluded*

EFFECT OF VARYING CONCENTRATIONS OF NITRATE AND NITRITE ON THE NITRITE TEST

[illegible]

of nitrate reduction by bacteria, particularly since positive results are significant, while negative results must be supplemented by further tests before an organism can be designated as a non-reducer. This point has been emphasized by Bronfenbrenner and Schlesinger (1), ZoBell (7) and particularly by Conn (2) who points out the need for caution in designating species as non-nitrate reducing. Use of a solution of too low concentration (e.g., 0.01 to 0.02% potassium nitrate) has doubtless been responsible for the classification, as non-reducing species, of organisms that may be nitrate-reducing, and has contributed to much of the confusion in the literature as to this characteristic.

The two species in Group B (Table II), a *Micrococcus* isolated from meat-curing pickle and a species of *Flavobacterium* isolated from the surface of a cow's udder, and causing ropy milk, represent less common types. These organisms reduce nitrates without causing disappearance of nitrite through further reduction or assimilation. Moreover the accumulation of nitrite in a medium containing but 0.001% potassium nitrate also suggests that none of the original nitrate is assimilated for cell building. In these respects the organisms appear to behave like *B. larvae*.

With the species in Group C no reduction is indicated, the persistence of nitrite in the medium with the lowest concentration of potassium nitrite indicating that the failure to show nitrite in the nitrate series is due to absence of reduction rather than to disappearance of nitrite.

(3) *Effect of Contaminants on Recognition of B. larvae and the Nitrite Test*

In the cultivation of *Bacillus larvae* from combs suspected of containing American foulbrood, or for the detection of viable spores remaining after treatment of diseased comb with disinfectants, surprisingly little trouble due to growth of contaminating organisms has been encountered. Occasional contamination does occur, due in the majority of cases observed, to the presence of spore-forming organisms of the *mesentericus* or *vulgatus* type. The whole trend of the cultural tests, however, suggests a possible antagonistic action between *B. larvae* and other organisms.

To examine the effect of association of *B. larvae* with other bacteria, three strains of the former, isolated from different sources, were each tested in association with eight other species. Of the latter, three are concerned with bee disease (*B. orpheus*, *B. alvei* and *Str. apis*), two were isolated as contaminants from diseased comb (*B. vulgatus*, *Bacillus* sp.) while the remaining three were other common types (*B. subtilis*, *Esch. coli*, *Staph. aureus*).

Tests were made in yeast-peptone-turnip semi-solid medium. This is similar to the yeast-peptone-carrot medium previously described (4) except that turnip extract is used in place of carrot, having been found to support better growth of *B. larvae*, and now employed in the routine examination for this organism. In one series the strain of *B. larvae* examined and the test organism were inoculated simultaneously, while in a second series the test organism was added 48 hours after *B. larvae*. Cultures were all held at 37° C. After 48 hours, examination was made for *B. larvae* in the first series, and

TABLE III
EFFECT OF ASSOCIATION OF *B. larvae* AND OTHER ORGANISMS

Organism	Nitrate broth, 0.1% KNO ₃	Nitrite broth, 0.002% KNO ₂	Yeast-peptone-turnip semi-solid medium								Yeast-peptone- turnip agar, antagonistic effect by <i>B. larvae</i>
			Inoculated singly		Simultaneous inoculation, <i>B. larvae</i> and test org.		Test organism inoculated 48 hr. after <i>B. larvae</i>				
			Growth	NO ₂	Growth of <i>B. larvae</i>	Growth of test org.	NO ₂	Growth of test org.	NO ₂		
<i>Controls</i>											
<i>B. larvae</i> (from worker scale)	No growth	No growth	+	+							
<i>B. larvae</i> (from drone larva)	No growth	No growth	+	+							
<i>B. larvae</i> (from queen cell)	No growth	No growth	+	+							
<i>Test Organisms</i>											
<i>B. subtilis</i> (246)	+	-	+	-	Slight	Good	-	Good	-	None	
<i>B. orpheus</i> (226)	+	-	+	-	Fair	Good	-	Fair	+	Definite	
<i>Esch. coli</i> (117)	+	-	+	-	Slight	Good	-	Good	+	None	
<i>Staph. aureus</i> (292)	+	-	+	-	Slight	Good	sl.	Good	+	None	
<i>B. vulgaris</i> (389)	-	+	+	-	Fair	Good	+	Slightly less	+	Slight	
<i>B. alvei</i> (127)	-	+	+	-	Slight	Good	+	Slightly less	+	None	
<i>Bacillus</i> sp. (390)	-	+	+	-	Fair	Fair	+	Slight	+	Slight	
<i>Str. aptis</i> (239)	-	+	+	-	Fair	Fair	+	Fair	+	None	

for comparative development of the test organism in the second series, the nitrite test being also made in all cases. In addition, cultures of *B. larvae* were made on yeast-peptone-turnip agar plates to which cultures of the test organisms were added, after 48 hours, to allow any visible antagonistic effect to be noted.

With each of the strains of *B. larvae* used results were similar, and these are summarized in Table III. In no case did simultaneous inoculation of the test organism prevent recognition of *B. larvae* microscopically, even though the contaminant developed well. Of the eight test organisms, four were nitrate-reducing and able to cause disappearance of 0.002% nitrite, and prevent accumulation of nitrite in the special medium when grown singly. Grown simultaneously with *B. larvae*, three prevented nitrite accumulation. The four other test organisms were non-nitrate reducing, and unable to reduce or assimilate small quantities of nitrite. They were unable to prevent growth of *B. larvae* or prevent accumulation of nitrite by the latter when grown in association.

Where the test organism was inoculated 48 hours after *B. larvae*, rather less development of the contaminant occurred, with the exception of *B. subtilis* and *Esch. coli*. After 48 hours' further incubation, nitrite accumulation by *B. larvae* was not detectable in the case of these two organisms only. With *B. orpheus* the positive nitrite test suggested an inhibiting action of *B. larvae*. This was confirmed by the results of the plate tests in which a definite antagonistic effect was observed. Less pronounced effect was observed with *B. vulgatus* and *Bacillus* sp. No. 390, and little or none with the other species tested.

No interference with the nitrite test for *B. larvae* in the special vegetable extract medium may be expected with non-nitrate-reducing contaminating species. These include not only most types associated with other brood diseases, but also the important *mesentericus* and *vulgatus* groups which may form the most frequent contaminating forms. Nitrate reducing species, e.g., *B. subtilis*, may interfere, though with *B. orpheus* disappearance of nitrite appears to be dependent upon the relative growth of the contaminant and *B. larvae*. It is important that no species tested prevented recognition of *B. larvae* by microscopic tests.

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COMPARATIVE STUDIES IN POTATO VIRUS DISEASES¹

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Abstract

This paper is an account of an investigation into the identity of a hitherto undescribed mosaic disease of President potato. Both a mild and a severe form of the disease were observed, but both forms were characterized by a yellow mottling not found in the previously reported potato mosaics.

The mild form of the disease has been shown to be caused by a single virus, while the severe form is due to a combination of this virus and one of the "vein-banding" group. Because of the yellow color associated with the symptoms produced on a number of host plants, the name "yellow mottle" is proposed for the newly described virus.

The "yellow mottle" virus of President mosaic has been compared with the "mottle" and "ringspot" viruses from rugose mosaic, both as to behavior under certain physical and chemical tests and with respect to the symptomatological reaction of a number of solanaceous host plants.

Tabulated results of the differential property studies are given, and descriptions of the symptoms caused by each of the three viruses on eight different host plants are presented.

From these studies it is concluded that the newly described "yellow mottle" virus is distinct from both "mottle" and "ringspot", but it is closely related to the "X-virus" or "latent virus" group.

Introduction

The potato variety known in England as "President" and in Germany as "Paul Kruger" has for a number of years been grown in Nova Scotia where it was given the name "Never Rot" because of its comparative freedom from late blight rot. It became quite widely grown and valued in those sections where blight injury was prevalent and was eventually entered for inspection under the Potato Certification Service of the Division of Botany, Experimental Farms Branch of the Dominion of Canada Department of Agriculture.

Inspection of the variety brought to light the fact that there existed in much of the stock a rather obscure mosaic disease (Plate I, Fig. 1) which could not definitely be identified with any of the named potato mosaics, although in its full development it bore a resemblance to crinkle and rugose mosaics. The disease appeared to be present in both a mild and a severe form, distinguishable from other types of mosaic by the characteristic yellow cast imparted to the foliage of the infected plants.

This report is based upon work performed in the Department of Botany of the University of Toronto, which was undertaken in order to investigate the nature of the mosaic disease of President and to determine the relationships of the virus or viruses concerned.

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Materials and Methods

Specimens of diseased potato tubers were obtained from various places at the beginning of the investigation, and during its progress a number of additional lots were obtained, largely duplicates from the original sources. The samples usually consisted of from three to six tubers, all of which were grown and examined in the course of the work.

Table I provides a list of the diseased specimens that were included in the comparative studies, together with the source from which they were received.

TABLE I

Source	Variety	Disease
Mr. W. K. McCulloch, Kentville, N.S.	President	Mosaic (mild)
	President	Mosaic (severe)
	Bliss Triumph	Mild mosaic
	Bliss Triumph	Rugose mosaic
	Green Mountain	Mild mosaic
	Green Mountain	Rugose mosaic
Mr. D. J. McLeod, Fredericton, N.B.	Green Mountain	Quanjer's crinkle
	Green Mountain	Mild mosaic
	Green Mountain	Rugose mosaic
	Bliss Triumph	Rugose mosaic
Dr. E. S. Schultz, U.S. Dept. Agr., Washington, D.C.	Green Mountain	Mild mosaic
	Green Mountain	Leaf rolling mosaic
	Green Mountain	Crinkle mosaic
	Green Mountain	Rugose mosaic

A number of other specimen tubers infected with named virus diseases were also received but were not included in the comparative studies beyond the preliminary stages.

Mr. McCulloch also kindly supplied healthy stocks of the four potato varieties: President, Green Mountain, Bliss Triumph, and Irish Daisy. Plants from these specimens and also from some of their progeny which were grown in Simcoe County, Ontario, were used in the inoculation studies.

In addition to the diseased potatoes, two other sources of virus were used in this investigation:

- i. Ordinary tobacco mosaic obtained from the Dominion Tobacco Experiment Station at Harrow, Ontario.
- ii. Veinbanding virus of tobacco from an infected Turkish tobacco plant kindly supplied by Dr. E. M. Johnson of the University of Kentucky.

The majority of the inoculation studies were performed upon solanaceous plants other than potatoes, the seeds of the non-commercial species of which were obtained through the seed exchange service of the Department of Botany.

The work was done in the plant houses of the Department of Botany, the houses having a temperature range of 15°–20° C. during the winter months, but somewhat higher during the spring and summer.

Inoculations, when made directly from plant to plant, were performed by the leaf-rubbing method. Flamed forceps were used to tear off leaflets or bits of leaf tissue, which were then rubbed lightly over the surface of the leaf to be inoculated. When plant extracts or filtered juices were employed, the rubbing was done with sterile forceps holding a small piece of sterile gauze. In some cases where the leaf-rubbing method failed or where infection seemed to be particularly slow, as in the inoculation of potato plants, grafting was employed.

The experiments with aphid transmission were performed in cages covered with cotton or with cotton and cellophane.

Experimental Procedure

PRELIMINARY

The first series of experiments was directed toward a comparison of the symptoms of different potato mosaics on a number of solanaceous hosts in order to determine (i) whether the reactions produced by inoculation with the various specimens of the same name were identical and specific, and (ii) whether the reactions produced by the virus from President mosaic would be specific and sufficient to differentiate it from the viruses of the other diseases.

The preliminary inoculations were carried out, using all the above sources of potato viruses, including apparently healthy potatoes, upon the following list of solanaceous hosts: *Nicotiana tabacum* var. Quesnelle, *Nicotiana glauca*, *Nicotiana rustica*, *Petunia hybrida*, *Physalis aequata*, *Nicandra physalodes*, *Datura stramonium* and *Lycopersicum esculentum* var. John Baier; from two to six plants were used in each case. All of these plants, in the seedling stage at least, were found to develop symptoms of virus infection when inoculated from any one of the diseased specimens.

It was further noted on all plants, but especially on *Nicotiana glauca*, *N. rustica*, *Datura stramonium* and *Lycopersicum esculentum*, that, when they were inoculated from the diseased President plants, the mottling differed in such a way as to be easily distinguishable from the mottling resulting when these plants were inoculated from other sources of potato viruses.

The chief diagnostic features of this mottling were a more severe yellowing of the intercostal areas of the leaf combined with a green banding effect along the veins. In those cases in which, as was later shown, mixtures of viruses were present, the definite pattern was obscured but the yellow color persisted in scattered patches.

In some cases inoculum from the healthy President plants produced results identical with that from other supposedly healthy potato plants, while in other cases no reaction at all was produced.

Plants inoculated from potatoes having rugose mosaic showed symptoms somewhat similar to those on plants inoculated from severely diseased President potatoes, except for the color difference noted above.

The difference between the mild and the severe disease of President potatoes was seen to be of the same order as the difference between mild and rugose mosaics of Green Mountain and Bliss Triumph; or, for that matter, between apparently healthy Green Mountain and those having rugose mosaic, since no difference in the symptoms upon the test plants could be seen whether they were inoculated from specimens having mild mosaic or from those which were apparently healthy.

It was therefore concluded that the diseased President potatoes contained a virus not found in any of the other diseased specimens and attempts were made to isolate this virus so that comparisons might be made.

FILTER ACTION OF *Datura stramonium*

K. M. Smith (10) and Koch (6) both reported that *Datura stramonium* was useful as a filter plant in separating the viruses found in some of the composite diseases of potatoes. It was therefore decided to experiment with the effect of passage upon *Datura stramonium*.

Accordingly, subinoculations were made from all the *Datura stramonium* plants that had been inoculated from the diseased potato plants in the first series of experiments.

The inoculations were made on the following test plants: *Nicotiana tabacum*, *N. alata*, *N. rustica*, *N. glutinosa*, *Hyoscyamus albus*, *Petunia hybrida*, and *Lycopersicum esculentum*; four or five plants of each species being used in each inoculation. Checks were provided by re-inoculations both from the original sources and from the other test plants used in the preliminary experiments.

Observations

No difference in the symptoms on the test plants could be distinguished when inoculations were made from *Datura stramonium* plants inoculated either from potatoes affected with mild mosaic or from apparently healthy potatoes. The same was true in the cases where the original inoculation had been from mildly diseased President potato plants.

In rugose mosaic, it was seen that passage through *Datura stramonium* had modified the symptoms on the test plants so that they were identical with those obtained by inoculation from apparently healthy Green Mountain potatoes.

The effect on the virus of the severe disease of President potatoes was similar, in that the symptoms obtained agreed with those resulting after inoculation from mildly diseased President plants.

The symptoms on all the test plants of any one species, inoculated from diseased *Datura stramonium* plants, were similar if the original sources of inoculum were from "mild mosaic", "crinkle mosaic", "Quanjer's crinkle", "leaf rolling mosaic", or "rugose mosaic". Moreover, they agreed very well with those described by Johnson (2) and Koch (6) in the case of the "mottle" virus from rugose mosaic and from apparently healthy potatoes.

The symptoms obtained when the original source of inoculum was diseased President potato plants were distinctly different in all cases from the above, but even after passage through *Datura stramonium* there were variations which suggested that the virus might still be mixed.

Similar experiments were performed with egg plants (*Solanum melongena* var. Black Beauty) in place of *Datura stramonium*, and it was found that the same differentiation of symptoms occurred.

FILTER ACTION OF PETUNIA

An attempt was made to duplicate the work of Smith (10) and to isolate a "Y" or "veinbanding" virus by means of the filter action of petunia. In all cases except one it was found that the mosaic complex was transmitted apparently unchanged.

In one case, however, a petunia plant inoculated from a severely diseased President potato plant failed to transmit the virus responsible for the typical yellow mottling on the test plants. Instead, it transmitted a virus which produced symptoms strongly suggestive of the "veinbanding" virus (12). A number of subinoculations were made from this source but the strain was unfortunately lost before complete identification could be made.

FURTHER ISOLATIONS FROM DISEASED POTATOES

Some months after the preliminary inoculations reported above, a set of duplicate inoculations was made. For the most part, the symptoms obtained agreed with those which had previously been observed. There were, however, notable exceptions. In three cases the symptom expression on the test plants was not that of the "mottle" virus as described by Johnson (2) but that of "ringspot". The sources of the inoculum were Green Mountain potato plants exhibiting symptoms of leaf rolling mosaic, crinkle mosaic and rugose mosaic.

The "ringspot" virus was found to be capable of passage through *Datura stramonium* plants and was in several cases obtained in pure culture, since subsequent attempts failed to demonstrate the presence of any other virus associated with it.

Differential Property Studies

From symptomatological evidence, it was considered that there had been recognized, in the course of the investigation, three distinct viruses which were capable of infecting *Datura stramonium*.

- i. The common "mottle" virus which is carried without symptoms by practically all American potato varieties.
- ii. The "ringspot" virus.
- iii. The "yellow mottle" virus of President mosaic.

The "mottle" virus is apparently often found without contamination in many varieties of potatoes without causing any symptoms; while the "ringspot" virus is apparently seldom found pure in the potato, although it was

accidentally isolated in the course of this investigation. It would seem that the distinctive virus of President mosaic is often found uncontaminated in that variety; but on the other hand, since the common "mottle" virus has also been found alone in President, and a considerable degree of irregularity in the symptoms upon test plants has been observed, it seemed probable that in many cases the ordinary "mottle" virus and the "yellow mottle" virus existed together. In order to establish the purity of the virus isolants, a number of property studies were carried out. The criterion upon which to judge the effect of any treatment is, of course, the symptom expression upon the test plants; and when a point is reached where no further modification takes place in the symptom expression, the virus under consideration is adjudged to be pure.

The tests performed consisted of attempts at insect transmission, investigation of the rate of spread through test plants, and studies on the resistance of the virus *in vitro*.

INSECT TRANSMISSION

Preliminary tests were made in order to determine whether any differentiation could be made on the basis of transmissibility by insects. The aphid *Myzus persicae* Sulz. was used in all the tests, healthy experimental stocks being secured by colonizing upon *Datura stramonium* or *Solanum melongena* in cotton cages.

Attempted transfers by means of aphids were made both from infected potatoes and from other solanaceous test plants, but in no case was infection obtained with any of the three viruses. As a check on the technique of these experiments aphid transfers of tobacco mosaic virus and "veinbanding" virus were also attempted and successfully carried out. It was therefore concluded that the "yellow mottle" virus of President mosaic resembled the "mottle" and "ringspot" viruses to the extent that it is not transmissible by the aphid *Myzus persicae* Sulz.

RATE OF SPREAD THROUGH THE PLANT

A number of experiments were performed in order to find out how long it takes after inoculation for the virus to reach the upper leaves of a plant. It was hoped that some difference in the rate of spread would be discovered that would help to differentiate the "yellow mottle" virus from the "mottle" and "ringspot" viruses. The plan of experiment in each case was to inoculate a plant on one of the intermediate leaves and then to make daily subinoculations from the tip of the plant until recognizable symptoms appeared.

In one case, the "yellow mottle" virus was obtained from the tips of tomato plants five days after inoculation, whereas definite symptoms of its presence were not visible until the seventh day. In another case, the virus was recovered from the tip of the plant on the eighth day, and the first symptoms were noted on the tenth day. In a similar test with *Nicotiana glutinosa*, the virus was recovered from the tip of the plant on the fifth day, while symptoms appeared on the seventh day.

The "ringspot" virus was obtained from the tips of tomato plants on the third day after inoculation, while the symptoms did not become apparent until the sixth day.

The "mottle" virus required from seven to nine days to reach the tips of inoculated tomato plants, and symptoms became visible in from ten to twelve days. Using *N. glutinosa*, the "mottle" virus was recovered from the tips of the plants in from five to eight days, whereas symptoms appeared in from seven to ten days, although in one case fifteen days elapsed.

The evidence afforded by these experiments would indicate that, in the tomato plant, the "ringspot" virus is able to move most rapidly through the tissues; the virus of President mosaic moves at an intermediate rate, while the ordinary "mottle" or "latent virus" is most sluggish in reaching the growing point.

RESISTANCE OF VIRUS EXTRACTS

The tests employed were filtration, dilution, exposure to heat and to various chemicals, and aging *in vitro*.

Filtration was carried out by passing the expressed plant juices through Mandler N candles. The juices were strained through gauze and passed through a pad of non-absorbent cotton to remove the coarser material before filtration. Strained plant extracts were also employed in the other tests.

The exposure to heat was carried out by placing about 2 cc. of a 1:5 dilution of the plant extract in a small thin-walled test tube fitted with a tight rubber stopper and completely immersing it in a water bath held at the required temperature for ten minutes. At the end of the ten minute period the test tubes were immersed in cold water until cool, and the inoculations were made immediately by rubbing with pieces of sterile gauze.

TABLE II

SUMMARY OF THE DATA ON FILTRATION, DILUTION END POINT, THERMAL DEATH POINT, AND INACTIVATING CONCENTRATION OF CHEMICALS

Test	"Mottle"	"Ringspot"	"Yellow mottle" virus of President mosaic
Filtration: Mandler N	X	X	X
Dilution end point	$\frac{1}{100,000}$	$\frac{1}{10,000}$	$\frac{1}{1,000,000}$
Thermal death point †	68-70° C.	66-68° C.	71-73° C.
Chemicals: ††			
Alcohol	50-75%	50-75%	50-75%
Nitric acid	$\frac{1}{500} - \frac{1}{400}$	$\frac{1}{500} - \frac{1}{200}$	$\frac{1}{500} - \frac{1}{200}$
Formalin	$\frac{1}{50}$	$\frac{1}{50}$	$\frac{1}{100}$
Phenol	2-4%	over 4%	2-4%

† 10 minutes exposure.

†† Concentrations which inactivate after one hour exposure.

The exposure to chemicals was also performed with a 1:5 dilution. The chemicals were all made up to double the required strength and then an equal quantity of the diluted plant juice was transferred by pipette into each test tube. The mixture was allowed to stand for thirty minutes when the first set of plants was inoculated by means of small pieces of sterile gauze, and at the end of an hour a second set of plants was inoculated in the same way.

Table II contains a summary of the data on the filtration dilution, heating and chemical treatments of the "mottle", "ringspot" and "yellow mottle" viruses.

On the whole, the records obtained in the property studies furnish very little basis for separation, with the exception of the thermal death points. Dilution tests are not to be relied upon, for little uniformity of result is ever obtained upon repeating an experiment. All three viruses seem to have equal powers of resistance toward inactivation by various chemicals. With respect to longevity, it was found that President mosaic virus retained its infectivity *in vitro* for more than two months, which compares favorably with the other two viruses. The evidence of these tests all points toward the inclusion of these three viruses as members of the same group.

THE BASIS OF SEPARATION

The problem of separation concerns only those residual viruses which are transmitted through *Datura stramonium* since by this passage the "vein-banding" viruses are eliminated. Koch (6) has pointed out that "ringspot" could be separated from "mottle" by virtue of the fact that it was capable of spreading more rapidly in the tissues of the tobacco plant. On the other hand, the "mottle" virus has a higher thermal death point and in this way the "ringspot" virus may be eliminated.

In this investigation similar differences have been found between these two viruses; the thermal death point for the "mottle" virus was 70° C. while that for the "ringspot" virus was 68° C.; the "ringspot" virus was able to reach the tip of a tomato plant in three days, while the "mottle" virus required six or seven days.

The higher thermal death point of the "yellow mottle" virus of President mosaic is enough to separate it from both "mottle" and "ringspot", as the virus which was found infective after heating to 72° C. caused no symptoms upon the test plants attributable to admixture with other strains. It has also been found that sometimes the virus of President mosaic is able to pass more quickly to the tip of an inoculated plant than is the "mottle" virus, but this difference is not constant.

The similarity in the responses of all three viruses to the various physical and chemical tests is evidence that these viruses are closely related. There is no difference to be found in the host range, as no test plant has yet been discovered that will retain any one of them to the exclusion of the others.

The ultimate justification of the differentiation of a complex into separate viruses lies in the fact that once one is obtained pure, by whatever means, the symptom expression on the test plants remains constant through successive subinoculations. All three viruses have remained constant through at least six successive transfers and have shown no tendency to change from one form to the other.

Symptomatology

The symptoms of the three potato viruses of the "latent" or "X-virus" group encountered in this investigation, upon each of eight different host plants, are described in the following paragraphs.

POTATO MOTTLE VIRUS

Tobacco (Nicotiana tabacum)

No primary symptoms were observed on the inoculated leaves of seedling plants, but vein clearing followed by a very mild mottling appeared on the young leaves in from two to three weeks. As the plants grew older the intensity of the mottling decreased until at flowering time no symptoms could be seen. No appreciable stunting of the plants took place.

Flowering Nicotina (N. alata)

Yellowish lesions appeared, in some cases, on the inoculated leaves in from ten days to two weeks, followed closely by vein clearing and a definite mottling of light and dark green, the darker color usually existing in patches along the veins. Neither primary nor secondary necrosis was seen. It was much more difficult to infect plants after they had passed the seedling stage.

Nicotiana rustica

Usually no primary symptoms were observed, although occasionally small lesions appeared at the point of inoculation. Clearing of the veins of the youngest leaves was observed about a week after inoculation, followed by a mild mosaic mottling of light and dark green areas without any sign of necrosis or distortion (Plate I, Fig. 2). The mottling faded out in the mature plants.

Nicotiana glutinosa

Primary symptoms were not observed. Secondary symptoms appeared in about ten days, the mottling being very mild and often almost unnoticeable (Plate I, Fig. 3). If the plants were inoculated as very young seedlings a slight stunting was noticed, but on older plants the effect was negligible.

Hyoscyamus albus

As a rule there were no primary lesions. Early secondary symptoms consisted of an indefinite clearing of the veins, while later symptoms consisted of a mild mottling sometimes accompanied by interveinal necrosis (Plate I, Fig. 4).

Petunia hybrida

No primary symptoms were observed, but mottling appeared in from two to three weeks. No stunting or necrosis was noted, the disease being of a very mild type.

Tomato (Lycopersicum esculentum)

A very mild mottling made its appearance in from one to three weeks, depending upon external conditions. As a rule the whole plant had a slightly yellowish or chlorotic appearance. The mottling usually consisted of dark green bands along the veins with light green intercostal areas. In older plants the symptoms tended to disappear, although a slight stunting sometimes occurred.

Jimson Weed (Datura stramonium)

No primary symptoms were seen but a faint mottling appeared in from five to ten days. This was usually of a mild veinbanding type which in some cases tended to become more intense (Plate I, Fig. 5).

POTATO RINGSPOT VIRUS

Tobacco (Nicotiana tabacum)

Small white-ringed necrotic lesions appeared on the inoculated leaves in from three to five days. The necrosis spread rather rapidly, especially at high temperatures, and often destroyed most of the tissue of the leaf. Secondary symptoms appeared on the upper leaves in from seven to ten days after inoculation and consisted of very numerous ringspots, each of which was about 1 mm. in diameter (Plate I, Fig. 6). These central rings became surrounded by larger rings and portions of larger rings, until very little green tissue was left. Later leaves were less severely affected, and in old plants the symptoms were reduced to a rather indefinite mottling interspersed with faint wavy lines. Tobacco seedlings were inoculated in April and kept in the greenhouses all summer, and in the fall no symptoms of any sort were exhibited but the virus was easily recoverable from the leaf tissues by inoculation upon other test plants.

Flowering Nicotine (N. alata)

Primary, white, necrotic ringspots appeared in five or six days. On young seedlings, the inoculated leaves soon became completely necrotic and dropped off. Secondary symptoms appeared in from one to two weeks after inoculation, usually beginning with clearing and necrosis of the younger leaves. This was followed by varying degrees of necrosis of the ring-and-line type (Plate I, Fig. 7). Later leaves usually displayed a milder type of necrosis and the upper part of the plant exhibited only a mottling which became less intense as the plant matured. Young plants were sometimes killed by systemic necrosis whereas older plants exhibited only a mild mottling in addition to the primary lesions on the inoculated leaf.

Nicotiana rustica

This plant was found to display the symptoms of ringspot to their best advantage. Primary symptoms sometimes appeared in less than three days after inoculation. At first they consisted of very small white rings on the inoculated surface and were not visible through the leaf. Within a day they deepened so as to be seen from the other side of the leaf and also became

surrounded by a second white ring (Plate I, Fig. 8). This process was repeated until the spots coalesced to form an irregular pattern of concentric lines. The inoculated leaf, especially in young seedlings, often became completely necrotic and was dropped. In older plants, only the inoculated portions of the leaf became necrotic. Secondary symptoms were noted on the young leaves, at the growing tip, in from one to two weeks after inoculation. Here the necrosis was usually in the form of an irregular ring-and-line pattern rather than small discrete rings as in the case of tobacco. Later, wide necrotic margins were noted along the principal veins of some of the leaves behind the growing point. The symptoms reached their full development on the intermediate leaves produced just subsequently to the systemic spread of the virus. Mottling, as generally understood, was not present. As a rule, bands of dark green tissue persisted along the chief lateral veins, but the intercostal areas were occupied by a zonate pattern of white necrotic lines, visible from both sides of the leaf (Plate I, Fig. 9). The upper leaves of these plants were usually symptomless or, at most, bore only a few small ringspots.

The younger the plant when it is inoculated, the more general is the necrotic effect of the virus. As the plant grows older it seems to develop some sort of resistance to the spread of the virus, for plants inoculated after the flower buds were formed produced only primary ringspots and partial necrosis of the inoculated leaf. That the virus did not become systemic was shown by the fact that it could not be recovered except from the inoculated leaf. In such cases, no immunity is conferred upon the rest of the plant, for the upper leaves of some of these plants were inoculated at a later date and primary lesions were produced.

Nicotiana glutinosa

Both primary and secondary symptoms on this plant resemble those of *N. rustica* except that there is a tendency for the secondary symptoms to be more irregularly necrotic, and to exhibit less of the ring-and-line pattern (Plate II, Fig. 10). In some cases it was found that the necrosis became systemic and killed the plants. The survivors exhibited a mottling, which gradually became less intense, and they were always very much stunted as compared with uninoculated control plants.

Hyoscyamus albus

Primary necrotic lesions appeared on the inoculated leaves in three or four days. In about two weeks a rather intense secondary necrosis was noted at the growing point, while at the same time discrete lesions appeared upon the intermediate leaves (Plate II, Fig. 11). The necrosis of the growing point gradually became less intense, and eventually the only symptom exhibited by the young leaves consisted of a mild mottling.

Petunia hybrida

No definite primary lesions were noted, but there were slightly discolored regions on the inoculated leaf. Secondary symptoms appeared very slowly as compared with those on other plants, but mottling and secondary necrosis

were observed about four weeks after inoculation. The plants sometimes recovered from the necrotic stage and produced mottled leaves.

The symptoms on petunia do not suggest the ringspots of other plants, but subinoculations produced typical symptoms on tobacco and *N. rustica*.

Tomato (Lycopersicum esculentum)

Primary local necrotic lesions were noted on the inoculated leaves in from three to five days, while vein clearing and fine necrotic spots appeared on the upper leaves within the week (Plate II, Fig. 12). These leaves always became necrotic and fell off; sometimes the whole top of the plant became necrotic and, in extreme cases, the plants died.

Usually, however, recovery takes place, and in about four weeks new leaves appear, which are only mildly necrotic but have a rather irregular mottling. (Plate II, Fig. 13). The symptoms gradually become less intense as the plant matures; the necrosis disappears, followed by the mottling, so that the upper parts of the plant may have the appearance of complete recovery. Subinoculations from the recovered parts, however, were found to transmit the virus in as virulent a form as ever.

Jimson Weed (Datura stramonium)

Small primary ringspots appeared on the inoculated leaves in six days. The inoculated leaves became completely necrotic and were dropped within a short time. The secondary symptoms depend a great deal upon the age of the plant. In seedlings, secondary necrosis may develop within three weeks and kill the plant. Older plants were almost completely defoliated, but put out new leaves exhibiting a rather intense yellow mottling with marked distortion and ruffling.

POTATO YELLOW MOTTLE VIRUS

Tobacco (Nicotiana tabacum)

The symptoms produced by this virus on the tobacco plant are not much more severe than those caused by the "mottle" virus. The mottling is somewhat more fine grained and may even tend toward half-rings in some cases (Plate II, Fig. 14). The small intercostal areas have a definite yellow color. As the plant matures the symptoms become less conspicuous, and old plants may be entirely symptomless. There is very little stunting.

Flowering Nicotine (Nicotiana glauca)

The primary symptoms usually consisted of yellowish lesions on the inoculated leaves, often followed by a diffuse necrosis. In about a week after inoculation the upper leaves exhibited a yellowish clearing of the veins, followed by mottling. In some cases the mottled leaves bore small necrotic spots. An extreme and very typical mottling was observed after about three weeks (Plate II, Fig. 15). It consisted of dark green bands along the veins and very light green or greenish-yellow intercostal areas. The mottling has an angular appearance not seen in the case of ordinary tobacco mosaic or other potato mosaic viruses. The mottling becomes much less conspicuous in older plants but was not observed to disappear entirely.

Nicotiana rustica

No primary symptoms appear on the inoculated leaves, but in a week or ten days necrotic spots appear on the young leaves at the growing tip. This is followed by a severe and definitely marked mottling with a few necrotic spots on the mottled leaves (Plate II, Fig. 16). As the plants mature the mottling becomes less severe and the young leaves do not exhibit necrosis.

Nicotiana glutinosa

Except for occasional slightly yellowed areas, primary symptoms do not appear on the inoculated leaves. Extreme clearing of the veins occurs in the young leaves in from eight to ten days, followed by a severe yellowish mottling of the same type as that observed upon *N. rustica* (Plate II, Fig. 17). Necrotic spots are frequently present, and the mottled leaves become senescent rather rapidly. The plants are usually very much stunted and often become almost completely defoliated.

Hyoscyamus albus

As a rule no primary symptoms were observed on the inoculated leaves. Secondary symptoms consisted of a mottling on the younger leaves which was usually somewhat more severe than that caused by the "mottle" virus (Plate II, Fig. 18).

Petunia hybrida

No primary symptoms were observed and the secondary symptoms were usually very slow in appearing. No necrosis occurred but the plants displayed a mild yellow mottling some weeks after inoculation (Plate II, Fig. 19).

Tomato (Lycopersicum esculentum)

Very specific effects were obtained with this virus upon tomato so that it was possible to distinguish it without difficulty from all the other viruses encountered.

In from five to seven days a mild yellow vein clearing appears on both the inoculated leaf and the young leaves at the top of the plant. In some cases there is necrosis of the young leaves, although usually the leaves showing clearing of the veins afterward expand normally and show the typical yellowish mottling. This mottling is much more extreme than the mottling caused by the "mottle" virus. The mottled leaves exhibit dark green bands of tissue along the veins while the intercostal areas are quite yellow, or in some cases bleached completely (Plate III, Fig. 20). If the plants are inoculated while very young, there is some tendency toward rugosity and rolling of the leaves, but in older plants there is no distortion. Badly infected plants are stunted and show little tendency toward recovery, although the symptoms become less distinct in mature plants.

Jimson Weed (Datura stramonium)

The first sign of disease is the clearing of the veins of the younger leaves, followed by a faint mottling. The mottling is sometimes produced by bands of dark green, but the most pronounced effect is usually a dark green spotting.

Later the mottling becomes more irregular, the chlorotic areas become yellow, and there is considerable distortion accompanied by small necrotic areas. The plants are stunted and the whole effect of the disease is more severe than after inoculation with either of the other two viruses (Plate III, Fig. 21).

Description of the Viruses

The communications of Johnson (3), Valleau and Johnson (12) and Koch (6) contain descriptions of the "mottle", "ringspot" and "veinbanding" viruses. In a recent publication, Johnson and Hoggan (4) have discussed the matter of virus identification and have indicated what they consider should constitute an adequate description of a filtrable virus. For the purposes of comparison their form will be followed in the summary descriptions of the three viruses isolated in the course of this work.

Potato mottle virus. Type: Johnson, J., Wisconsin Agr. Expt. Sta. Res. Bull. 63, 1925. Not transmissible by means of the aphid *Myzus persicae* Sulz. Readily transmissible by means of needle, tissue rubbing or graft inoculation. Filtrable readily through Mandler N and Berkfeld W candles. Longevity *in vitro*, several months. Thermal death point 70° C., for ten minutes. Host range: Potato (*Solanum tuberosum* L.), Tobacco, (*Nicotiana tabacum* L.), Nicotine (*N. alata* L.), Jimson weed (*Datura stramonium* L.), and a great many other members of the Solanaceae. Symptomatology: Mild mottling of green and light green shades on the foliage of most solanaceous plants in the younger stages. In older plants the mottling is often obscured. In most American potato varieties the virus is carried without symptom expression. Distribution coexistent with potato culture.

Potato ringspot virus. Type: Johnson, J., Wisconsin Agr. Expt. Sta. Res. Bull. 63, 1925. Not transmissible by means of the aphid *Myzus persicae* Sulz., but readily transmissible mechanically by means of plant juices or by grafting. Filtrable readily through Mandler N and Berkfeld W candles. Longevity *in vitro*, several months. Thermal death point, 68° C., for ten minutes. Host range: Potato (*Solanum tuberosum* L.), Tobacco (*Nicotiana tabacum* L.), Nicotine (*N. alata* L.), *N. rustica* L., *Datura stramonium* L., and many other solanaceous plants. Symptomatology: Distinctive, small ring-shaped necrotic spots at the point of inoculation on tobacco, *N. alata*, *N. rustica*, and *Datura stramonium* followed in a few days by the development of ringspots on the upper leaves of the plants. Best characterized by the development of a remarkable ring-and-line pattern on the leaves of *N. rustica*. In most American potato varieties this virus is carried without symptoms. Distribution coexistent with potato culture although not so frequently encountered as the potato mottle virus.

Potato yellow mottle virus (President mosaic virus). Type: this paper. Not transmissible by the aphid *Myzus persicae* Sulz. but readily transmissible mechanically by plant extract and by grafting. Filtrable readily through Mandler N and Berkfeld W candles, and with difficulty through Seitz E. K.

size 3 discs. Longevity *in vitro*: two months or more. Thermal death point: 73° C. for ten minutes. Host range: potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), Nicotiana (*N. glauca* L.), tomato (*Lycopersicon esculentum* L.), Jimson weed (*Datura stramonium* L.), and many other solanaceous plants. Symptomatology: slightly yellowish interveinal mottling on President potato, and an indefinite mottling on tobacco, but best characterized by a strikingly regular mottling on tomato in which there are bright yellow interveinal areas and very dark green bands of tissue along the veins (Plate III, Fig. 20). Distribution: reported only from the variety President grown in Nova Scotia.

Re-inoculation and Re-isolation

In order to complete the investigation it is necessary to reproduce the original disease by re-inoculation of healthy specimens of the original host with the isolated viruses, and then to re-isolate the same viruses. This is difficult, for obvious reasons, and doubly so when the original host is the potato which may contain so many "latent" or hidden viruses, each of which may be able to influence the reaction.

RE-INOCULATIONS ON POTATO PLANTS

The plants used in the tests were grown from tubers obtained from Nova Scotia as healthy, or from their progeny grown in tuber units in Ontario. Each tuber unit used was tested for the presence of "latent" viruses. In all cases, Green Mountain, Bliss Triumph and Irish Daisy contained the "mottle" virus while the President plants apparently contained no sap-transmissible viruses. The following is a record of the series of re-inoculations on potato.

1. Rugose mosaic was inoculated into apparently healthy Green Mountain plants directly from Green Mountain potatoes, and after passage through tomato, tobacco *Nicotiana glauca*, petunia, and *Datura stramonium*. At the same time, direct inoculations were made from an apparently healthy Bliss Triumph potato and from mottled tobacco and tomato plants which had been inoculated from it.

The plants inoculated with rugose mosaic, both directly and after passage through tomato, tobacco, *N. glauca* and petunia, all developed severe current-season symptoms of rugose mosaic and died within a month. The potato plants inoculated from apparently healthy Bliss Triumph, or test plants inoculated from it, remained apparently healthy, as also did those inoculated with rugose mosaic after passage through *Datura stramonium*.

2. A number of attempts have been made to inoculate Green Mountain and Bliss Triumph potatoes with the mosaic from President potatoes, both directly and after passage through various test plants, but in no case have any symptoms been observed. In nearly all cases attempts were made to re-isolate the virus from the inoculated plants, but to date there is no evidence that Green Mountain or Bliss Triumph potatoes either become diseased or act as symptomless carriers toward the virus from President potatoes

3. The "ringspot" virus from diseased tomato plants was inoculated into four Green Mountain, four Bliss Triumph, and four President plants. No apparent symptoms were obtained upon the inoculated plants. Attempts at re-isolation of the virus were made by taking a leaf from each of the four plants in each series, macerating them together, and inoculating two *Nicotiana rustica* plants from each series. Mild "ringspot" symptoms were obtained in all cases, and in addition, typical "mottle" symptoms were obtained from the Green Mountain and Bliss Triumph varieties.

4. Twelve President potato plants were inoculated with the virus of President mosaic after passage through *Datura stramonium*. All of them became diseased and exhibited a mottling identical with that of the original specimens. The tubers from one of these plants were grown again in the greenhouse and exhibited typical symptoms.

5. Eight President potato plants were inoculated with the virus obtained by passage of the President mosaic through petunia. These plants all showed a mild mottling about a month after inoculation, rapidly followed by a yellowing or premature senescence of the whole plant, which soon succumbed.

6. Four President potato plants were inoculated with a combination of the virus of President mosaic after passage through *Datura stramonium*, and the virus obtained by passage through petunia. The typical President mottling appeared first in the tip leaves of all the plants. Following that, three of the plants began to turn yellow and succumbed in the same manner as those inoculated with the petunia virus alone.

7. "Veinbanding" virus from the tobacco plant supplied by Dr. E. M. Johnson was inoculated upon two plants each of the President, Bliss Triumph, Irish Daisy, and Green Mountain varieties. Infection was obtained on all the inoculated potatoes with the exception of one President plant. The symptoms upon President consisted of necrosis and abscission of the inoculated leaf followed in about three weeks by the appearance of pale patches on the upper leaves. In the case of Bliss Triumph variety a mottling appeared in the upper leaves of both plants, followed by a yellowing and necrosis of the leaves much like that observed when President plants were inoculated with the virus obtained by passage through petunia. The symptoms resembled those reported by previous investigators. The symptoms on the Green Mountain and Irish Daisy varieties were similar.

RE-ISOLATION FROM ARTIFICIALLY INOCULATED POTATO PLANTS

Re-isolations on test plants were attempted in all the foregoing cases in which the viruses were re-inoculated on potato. In only one case, however, was it possible to demonstrate, by the re-isolation of the virus in a pure state that a typical mosaic disease had been caused by the inoculation of a potato plant with a single virus. In all other cases, either viruses were recovered from apparently symptomless plants, or else those plants which exhibited symptoms were shown to contain virus mixtures.

The President mosaic virus used in the re-inoculation on potato was obtained from a tomato plant which exhibited very characteristic and unmistakable symptoms. After symptoms had appeared on the potato plants, a series of re-isolations were made on tomato plants which exhibited the same typical mottling without any modification.

Thus it is possible to present a complete chain of evidence, including isolation, re-infection and re-isolation, to show that a single virus entity is able, unassisted, to cause a specific mosaic disease on the President variety of potatoes. This can be done, only because of the fact that there exist stocks of the President variety that are free from the "latent" viruses.

The Recombination of Separate Viruses into Artificial Associations

COMBINATIONS CONTAINING THE "VEINBANDING" VIRUS

Since the rugose mosaic complex was found to produce a standard necrotic disease on *Nicotiana alata*, it was deemed advisable to make comparisons on this plant by inoculation with combinations comprising the "veinbanding" virus and each of the three residual viruses. The reactions of the test plants are given in Table III. One set of plants was inoculated directly with rugose mosaic from a potato plant as a control. The two viruses in each combination were rubbed on opposite halves of the same leaf on each plant.

TABLE III
RESULTS OF INOCULATION OF *Nicotiana alata* WITH VIRUS COMBINATIONS

Inoculum	The reaction on four <i>N. alata</i> seedlings
Rugose mosaic (control)	4 plants, vein clearing and necrosis
"Veinbanding" and "mottle"	4 plants, vein clearing and necrosis
"Veinbanding" and "ringspot"	3 plants, vein clearing and necrosis 1 plant, ringspot only
"Veinbanding" and "yellow mottle" virus from President mosaic	4 plants, vein clearing and necrosis

The symptoms of the combination diseases appeared much sooner than did the vein clearing upon *N. alata* plants inoculated with the "veinbanding" virus alone. The plants inoculated with "ringspot" in combination with the "veinbanding" virus exhibited symptoms a day earlier than the other combinations, but all showed symptoms within a week after inoculation.

The clinical picture of the early stages of the disease was much the same on all the plants, and resembled closely that observed on *N. alata* seedlings when inoculated from severely diseased President potatoes. Complete necrosis of the central leaves together with a partial necrosis along the veins of older leaves took place in all cases (Plate III Figs. 22-25). In later stages, those plants which were inoculated with combinations containing either the "ringspot" virus or the "yellow mottle" virus from President mosaic appeared to be more severely diseased.

The success of these experiments in synthetically reproducing the original disease picture in each case is conclusive evidence that the severe disease of President was caused by a virus complex. It is also further evidence of the relationship of the residual virus of President mosaic to the group of "latent" or "X-viruses".

TOMATO STREAK

Interest in the "streak" disease of tomatoes was aroused quite early in this investigation by the discovery of a typically diseased tomato plant in a group which had been inoculated from diseased President potato plants. The symptoms exhibited by this plant agreed quite well with those described by Vanterpool (13), and it was thought that the disease had been caused by accidental contamination from an adjoining house containing plants infected with tobacco mosaic. Accordingly an attempt was made to analyze the complex by inoculation on *Nicotiana glutinosa*, which successfully eliminated the tobacco mosaic virus. At the time, although it was not certain that pure cultures of the various potato mosaic viruses had been isolated, a preliminary inoculation series was performed in which twelve different isolations from diseased potato plants were separately combined with the virus of tobacco mosaic, and inoculated on tomato plants which were about a foot high. All the plants became diseased and showed "streak" symptoms varying from a slight degree of wilting to systemic necrosis and death of the plant. A number of experiments were performed in the hope of determining the cause of this great variation, but without success.

At a later date, after the identity of all three separate residual viruses from potatoes had been established, further experiments were undertaken in order to study the effect of combining each of these residual viruses with the tobacco mosaic virus upon the tomato plant. It was found that all three combinations produced a typically necrotic disease on tomato. The disease produced by the combination of tobacco mosaic virus and the ordinary "mottle" virus, however, was slightly less virulent than the other two. This, of course, might be expected, as both the "ringspot" and the "yellow mottle" viruses are able at times to cause necrotic symptoms on the leaves of tomato.

The symptoms of tomato "streak" have been well illustrated by other investigators, but as this record is an addition to the number of viruses that can be combined with the virus of tobacco mosaic to cause "streak", photographs of symptoms are appended (Plate III, Figs. 26, 27). Those plants which recovered from the necrotic phase of the disease exhibit a strikingly yellow mottling and rugosity of the foliage; occasionally also, leaflets appear upon which are exhibited symptoms typical of one or other of the component viruses.

It was noted also, when this mixture of viruses was inoculated on tomato plants already exhibiting the "mottle" disease, that the symptoms were not so severe, and the recovery was more rapid than in the case where the inoculation was made on healthy plants.

The fact that all three residual viruses are able, in combination with the virus of tobacco mosaic, to cause typical "streak" of tomatoes is further evidence that they are related. The evidence that "streak" is less virulent if the tomato plants are already infected with the "mottle" virus, is in agreement with the statement of Ainsworth (1), and might be said to indicate a relationship between the potato virus fractions of the "streak" virus.

COMBINATIONS OF RESIDUAL VIRUSES

In the preliminary experiments there was evidence to indicate the existence in potato plants of natural mixtures containing the "mottle" and "ringspot" viruses, as well as the "mottle" and "yellow mottle" viruses. It was felt that an attempt should be made to reconstitute these observed mixtures from isolated viruses; and, although such a mixture had not been detected, a similar attempt was made to produce a combination of the "ringspot" and "yellow mottle" viruses.

Tomato plants about a foot in height were used for the experiment and the inoculations were made simultaneously with each pair of viruses on opposite halves of the terminal leaflet of an intermediate leaf.

In order that a complete series of reactions might be observed, the following combinations were employed; "mottle" and "ringspot", "mottle" and the "yellow mottle" virus from President mosaic, and "ringspot" and the "yellow mottle" virus. As soon as the first symptom became apparent subinoculations were made from the inoculated leaflets and from the tip of the plant in each case. The symptoms which developed upon the upper parts of the plants, and upon the plants subinoculated from their tips, showed that both viruses became systemic in each case. On the other hand, the viruses remained unmixed in the inoculated parts.

The symptoms, in the "mottle" and "ringspot" combination, consisted of a mild mottling, indistinguishable from that observed on tomato plants infected with the "mottle" virus, or the later stage of the "ringspot" disease. Subinoculations upon *Nicotiana rustica* proved that the "ringspot" virus was present with the "mottle" virus.

In the two other combinations an irregular yellow and green mottling was caused on the upper parts of the plant and on the plants subinoculated from their tips (Plate III, Fig. 28). The mottling was slightly more intense on the plant inoculated with the combination of "ringspot" and "yellow mottle" viruses. The "ringspot" virus was obtained in a pure state from this combination by means of needle inoculation from the green portions of the leaf; while rubbing with bits of crushed tissue transmitted a combination disease. Unfortunately, it was not possible to make a study of the localization of the viruses in other cases.

In another attempt to synthesize a combination disease, two tomato plants were inoculated with the "mottle" virus; and after systemic symptoms had appeared, they were again inoculated with the virus from President. The later symptom expression of both plants was changed and an irregular yellow mottling appeared in place of the mild green mottling caused by the first

inoculation. Subinoculations from these plants reproduced the irregular symptoms upon other tomato plants, but it was noted that the mottling became somewhat more fine-grained and approached quite closely in appearance to the symptoms produced by direct inoculation from mildly diseased President potato plants.

Discussion

From the above experiments and observations it is evident that the diseased President potato plants were infected by a virus differing from any that could be found in the standard named types of potato mosaics that were available for comparison. From its behavior in regard to insect transmission and passage through *Datura stramonium* it is evidently related to the "mottle" and "ringspot" viruses of Johnson (2) and Koch (6), or the "latent" and "virulent latent" viruses of Jones, Anderson and Burnett (5). In its ability to produce a mosaic disease of potato alone and unaided, it shows affinities with the "simple mosaic" virus of Murphy and M'Kay (8). Its behavior under exposure to certain physical and chemical tests is an additional reason for regarding it as a member of the group which may be termed after Smith (10) "X viruses".

This investigation has produced no evidence to show that any of the viruses of this group may change form by passage through different host plants. On some hosts the symptoms are indistinguishable, but upon transfer to a suitable host the distinguishing symptoms always reappear. Certain plants have been found to exhibit distinctive symptoms which are specific for the identification of certain viruses; the symptoms of "ringspot" appear most characteristically on *Nicotiana rustica* while those of the "yellow mottle" virus of President mosaic are most fully developed on the tomato. It may therefore be necessary to work with a fairly extended list of host plants in order to differentiate completely all the members of a virus group. The difference in the severity of the symptoms between the mild and severe types of the mosaic disease of President is of the same order as that shown in the case of the mild and rugose mosaics of Green Mountain potatoes. Koch (6) has shown that rugose mosaic is a combination disease caused by infection with either the "mottle" or the "ringspot" virus and a "veinbanding" virus. Schultz, Bonde and Raleigh (9) report that mild mosaic is caused by an aphid-borne virus and the "latent mosaic virus". The difference between the two diseases is therefore due to the difference between the two aphid-borne viruses. In the severe disease of President potatoes it was found that a virus of the "veinbanding" type was present, but the mild disease is caused by a single virus. The condition observed in the President variety in Nova Scotia is parallel to that described overseas where Murphy and M'Kay (8) have shown that the "crinkle" of Murphy (7) is caused by a compound infection with the virus of "simple mosaic" and "virus A".

Experimentally at least, tomato streak may be caused by a combination of the "yellow mottle" virus with tobacco virus 1; this is further evidence of its relationship with the "latent" viruses. The behavior of the "yellow mottle"

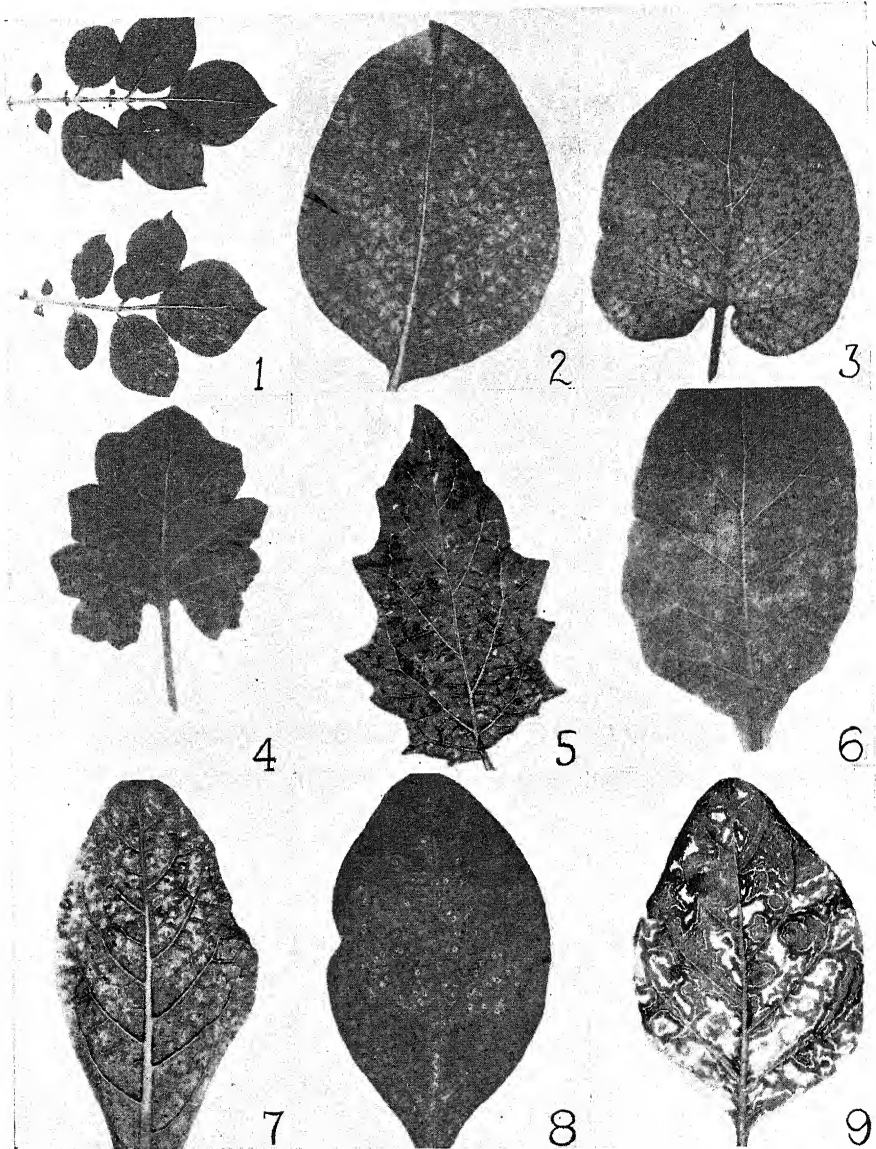


FIG. 1. Healthy and diseased leaves of President potato. FIG. 2. Leaf of *Nicotiana rustica* infected with the "mottle" virus. FIG. 3. Leaf of *N. glutinosa* infected with the "mottle" virus. FIG. 4. Leaf of *Hyoscyamus albus* infected with the "mottle" virus. FIG. 5. Leaf of *Datura stramonium* infected with the "mottle" virus. FIG. 6. Tobacco leaf about ten days after inoculation with the "ringspot" virus. FIG. 7. Typical symptoms of "ringspot" upon an intermediate leaf of *N. alata*. FIG. 8. Leaf of *N. rustica* three days after inoculation with the "ringspot" virus. FIG. 9. Fully developed symptoms of "ringspot" upon an intermediate leaf of *N. rustica*.

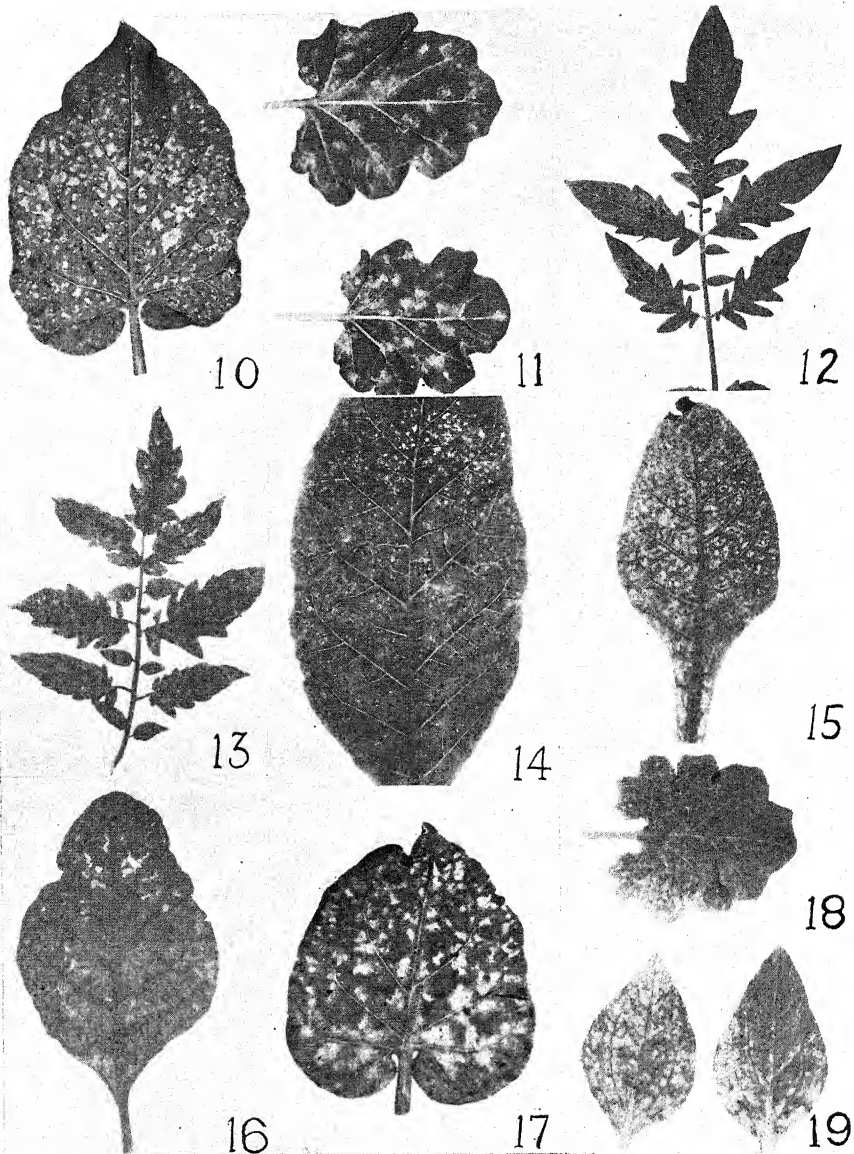


FIG. 10. Leaf of *N. glutinosa* showing symptoms of "ringspot". FIG. 11. Leaves of *Hyoscyamus albus* showing symptoms of ringspot. FIG. 12. "Ringspot" symptoms on a young tomato leaf. FIG. 13. Tomato leaf showing symptoms of a later stage of the "ringspot" disease. FIG. 14. Leaf from tobacco plant infected with the virus from President potato. FIG. 15. Leaf of *Nicotiana glauca* about three weeks after inoculation with the virus from President potato. FIG. 16. Leaf of *N. rustica* infected with the virus from the President potato. FIG. 17. Leaf of *N. glutinosa* infected with the virus from President potato. FIG. 18. Leaf of *Hyoscyamus albus* infected with the virus from President potato. FIG. 19. *Petunia* leaves showing symptoms of infection with the virus from President potato.



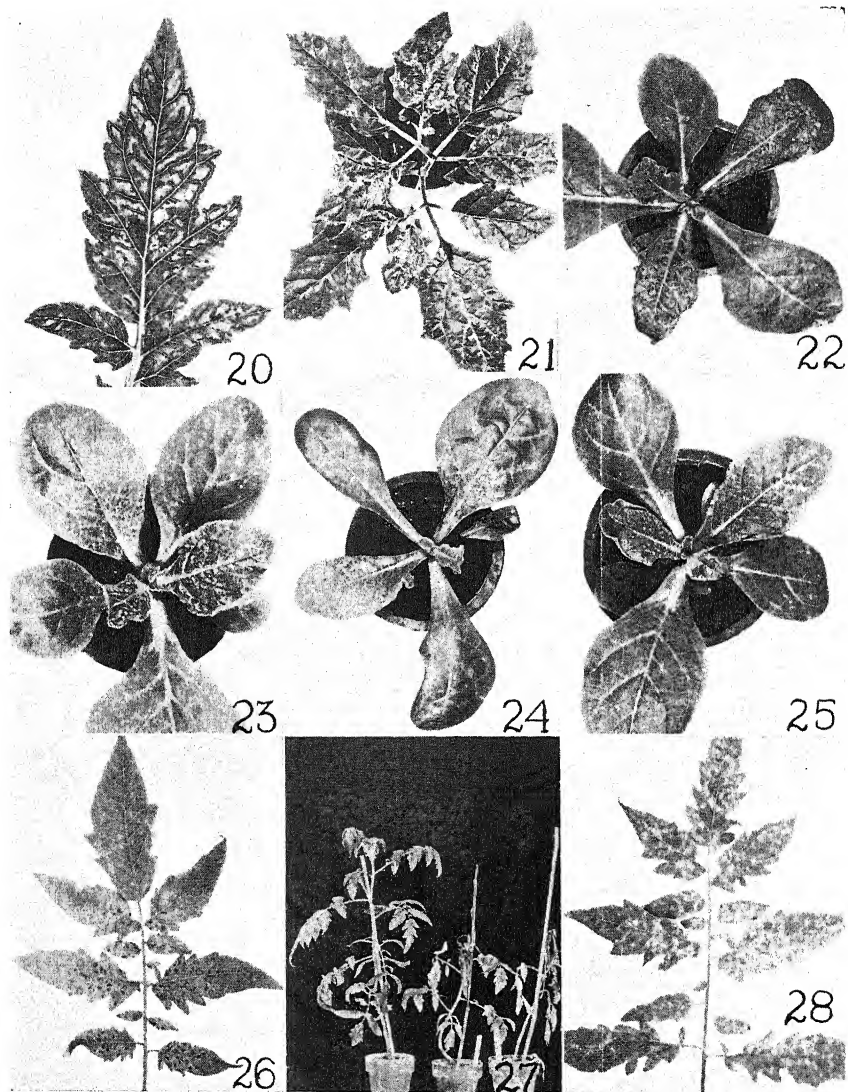


FIG. 20. Leaflet from tomato plant showing symptoms of infection with the virus from President potato. FIG. 21. *Datura stramonium* plant infected with the virus from President potato. FIG. 22. *Nicotiana glauca* plant ten days after inoculation with rugose mosaic. FIG. 23. *N. glauca* plant ten days after inoculation with the "veinbanding" and "mottle" viruses. FIG. 24. *N. glauca* plant ten days after inoculation with the "veinbanding" and "ringspot" viruses. FIG. 25. *N. glauca* plant ten days after inoculation with the "veinbanding" and President mosaic viruses. FIG. 26. Tomato leaf showing early symptoms of "streak". FIG. 27. Tomato plants showing "streak" and healthy control. FIG. 28. Leaf of tomato plant inoculated simultaneously with the viruses of "ringspot" and President mosaic.

virus when inoculated into the tomato simultaneously with either the "mottle" or "ringspot" viruses is in line with the findings of Ainsworth (1) and is a further indication of relationship. It does not, however, explain the fact that both "mottle" and "ringspot" viruses exist simultaneously in the same potato plant, as also may the "mottle" and "yellow mottle" viruses.

Conclusion

The distinctive symptoms observed in the mosaic disease of President potatoes are due to the presence of a hitherto undescribed virus, which differs from both the "mottle" and the "ringspot" viruses in symptomatology upon test plants, especially in producing a yellow mottling on tomato and some other species. They also possess a number of points of similarity. All three viruses are able to infect the same range of host plants and can be separated from the "veinbanding" virus by passage through *Datura stramonium*. They have similar resistance to chemicals and have only slightly separated thermal death points. In combination with other viruses such as the "veinbanding" virus or tobacco virus 1 they cause similar symptoms on a number of host plants, especially in spot necrosis on nicotine or streak of tomato. The "yellow mottle" virus of President mosaic is therefore to be considered as an additional member of the "latent" or "X-virus" group.

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THE PARASITISM OF *CLADOSPORIUM FULVUM* COOKE AND THE GENETICS OF RESISTANCE TO IT¹

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Abstract

Four physiologic forms of the imperfect fungus *Cladosporium fulvum* Cooke, causal agent of the leaf mold disease of tomatoes, have been differentiated by differences in pathogenicity. Cultural studies likewise have shown that this species is a composite of physiologic forms.

Saltant strains of differing degrees of stability have been isolated repeatedly from cultures arising from single one-celled spores. Since each cell of a conidium contains a single nucleus, these saltant strains are considered to arise as a result of mutations, in the broad sense of the term.

Four main classes of reaction to *C. fulvum* have been defined: complete susceptibility, two types of partial resistance, and immunity.

The reaction between pure lines of host and parasite is plastic. Environmentally conditioned variations in each of the four reaction types have been described. Of such variations the seasonal fluctuations in the reaction of *Lycopersicon esculentum* var. Stirling Castle to Form 1 are outstanding. It has been shown that the failure of the expression of the inherent resistance of this variety during midwinter at Toronto is due largely to the reduced light experience of plants grown at this time, while the failure of such plants to support sporulation is caused by the low relative humidity then prevalent in the greenhouses.

The genetics of the three types of resistance was fully analyzed. The Red Currant tomato, *L. pimpinellifolium*, carries, in addition to the dominant factor for immunity, an independently segregating dominant factor which, in the absence of the immunity factor, governs resistance to all four forms of *C. fulvum*. The resistance of Stirling Castle to Forms 1 and 3 has been shown to be due to another dominant factor.

Conspicuous among the genetic factors in the host which modify the main reaction types is the recessive lutescence factor in the homozygous condition. Its most striking effect is the production, on genetically immune individuals, of small inconspicuous infection spots whose increase in size is arrested very soon after symptoms appear.

As a result of linkage studies the three resistance factors have been located in MacArthur's (12) chromosome maps of the tomato.

The conflicting reports concerning the resistance of esculentum tomato varieties to *C. fulvum* are discussed in the light of physiologic specialization and of a plastic host reaction.

Introduction

During the last decade, a voluminous literature has been built up around the leaf mold disease of tomatoes, but most of the reports have dealt with rather empirical methods of control. In many quarters, however, control through the use of resistant varieties has been sought, and Guba (9) has summarized the conflicting reports of fourteen investigators concerning the occurrence of resistance among ten of these varieties.

The present investigation began with a study of the linkage relations of the factor for immunity to *C. fulvum*, found in the Red Currant tomato. This led to the discovery of additional types of resistance and of physiologic specialization and to a study of the variability of *C. fulvum* and of the plasticity of host-parasite relations.

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Materials and Methods

In the early stages of the investigation, isolations from diseased plants were made by plating bits of infected leaf tissues which had been surface sterilized with mercuric chloride. After familiarity with the appearances of cultures of all ages was acquired, it was found safe and convenient to make direct transfers from fruiting lesions to agar slants, using a sterilized needle. Nearly all cultural studies were carried out on slants of potato-dextrose agar containing 2% dextrose and 2% agar.

Conidia for inoculation purposes were secured either from artificial cultures or from infected leaves. In the latter event the infections were produced by a strain which had been in culture previously and whose pathogenic capabilities were known. Conidial suspensions were made in tap water or in distilled water. Germination tests were commonly carried out in the same media, although it was shown that vigorous germination and growth occurred in normal decoctions (17) of the immune Red Currant tomato as well as in decoctions of resistant and susceptible esculentum varieties. It was observed frequently, in connection with germination tests, that spores capable of prompt and vigorous germination lost this ability after the suspension had been shaken for from five to ten minutes but regained it when the water was allowed to evaporate from the suspension, the spores germinating when again provided with conditions of high relative humidity. In general, vigorous plants from one to two months old, grown in four-inch pots, were used for inoculation purposes. In connection with studies of the inheritance of resistance, plants of the resistant variety were always included in the inoculations and F_1 plants were frequently tested along with F_2 plants. For actual inoculation the plants were removed from the greenhouse to avoid needless mixing of strains, and immediately after its preparation the suspension was applied to the lower surfaces of the leaves with a DeVilbiss atomizer. After the plants had stood for about an hour they were transferred to an inoculation chamber in which the relative humidity was maintained close to saturation. Inoculations were usually carried out in the evening and the plants were removed from the inoculation chamber the following evening.

When the securing of linkage data involved growing plants to maturity, e.g., for the observation of fruit colors, pathogenicity tests were carried out before the plants were transferred from the greenhouse to the field; in some instances further tests were carried out on cuttings from the plants in the field. Immer's (10) tables were used for the calculation of linkage intensities.

Material for the study of the nuclear situation in conidia was fixed for 24 hours in Bouin's fluid and stained with gentian violet according to Smith's (20) method.

Experimental Results

STUDIES OF PHYSIOLOGIC SPECIALIZATION

The existence of physiologic forms of *C. fulvum* has been demonstrated by differences in the pathogenicity of various cultures. Tests involving 60 varieties revealed only one differential reaction, and of six esculentum varieties

that gave this reaction, Stirling Castle was chosen for subsequent studies. Stirling Castle is completely susceptible to some cultures of *C. fulvum* but, as a result of inoculation with others, gives a resistant reaction which, on young vigorous plants under conditions of abundant light and high relative humidity, is as follows. Symptoms appear in about the same time as on such susceptible varieties as Potentate but differ from them from the outset. The first whitish coloration due to the outward growth of fungous hyphae through the stomata of the leaf is restricted to a much smaller region than on Potentate. This restriction of development continues and, whereas in a few days infections on Potentate have spread considerably and turned brown in the centre owing to spore production (Plate I, Fig. 1, A), infections on Stirling Castle are not sporulating but appear as a compact whitish growth which is usually raised and puckered at the centre (Plate I, Fig. 1, B). At this stage infections on vigorous Potentate plants have not caused any conspicuous change in the appearance of the upper surface of the leaf opposite the infections, but those on Stirling Castle have caused a conspicuous yellowing of this surface. Whereas a heavy infection usually kills entire leaves on Potentate within a month after inoculation, comparable leaves on Stirling Castle are not killed but have, around the centres of infection, yellow spots which are more conspicuous on the upper than on the lower surface of the leaves. By this time many of these infections show a slight amount of sporulation, restricted to an area of from one to two millimetres in diameter at the centre of the spot.

In the pathogenicity tests to determine the extent of physiologic specialization 22 isolations of *C. fulvum* from 11 localities, one at Macdonald College, Quebec, the remainder in central and southwestern Ontario, were used. On Stirling Castle 15 of these produced the resistant reaction described above; the remaining 7 produced a reaction entirely comparable with the susceptible reaction on Potentate (Plate I, Fig. 2, B). Strains characterized by the production of a susceptible reaction on Potentate and of a resistant reaction on Stirling Castle are designated physiologic Form 1, and strains producing a susceptible reaction on both Potentate and Stirling Castle are designated physiologic Form 2 (Plate I, Fig. 2). Form 1 was obtained as follows: six times from Vineland Station, once from Macdonald College, Quebec, and once from the following localities in Ontario: Beamsville, Burlington, Colborne, Grimsby (two collections), Owen Sound, Simcoe and Vineland. Form 2 was obtained five times from Vineland Station and once from Beamsville and Vineland.

Since at Vineland Station, the only locality in which a number of collections were identified, five out of 11 cultures were of Form 2, it is believed that a study of a larger number of cultures would demonstrate a much wider distribution of this form than is indicated above.

Several preliminary inoculations indicated that symptoms differing from those produced by Forms 1 and 2 were in some cases the results of infections by mutant strains of the fungus. Accordingly a comprehensive inoculation

was made on September 4, 1934, under conditions of relative humidity enabling the development of sporulating infections. Vigorous uniform four-weeks-old plants of Potentate, Stirling Castle and five other varieties were inoculated in duplicate with 12 cultures of the fungus, including four known mutant strains which had been subcultured several times and six recently isolated cultures which had shown no evidence of mutation. The initial symptoms of infection on each variety were similar in the 12 series, but in some series, instead of the fungus growing out from the infected spots and sporulating, a progressive necrosis of the infected tissue occurred which, on susceptible varieties, resulted in enlarging dried-out spots resembling those following inoculation with "normal strains"* of the fungus under conditions of low relative humidity (Plate II, Fig. 4). Reactions characteristic of Forms 1 and 2 were produced by five cultures, including four of those recently isolated. Three cultures, including two of the recent ones, produced a mixed reaction of sporulating and non-sporulating infections. The four mutant strains gave rise exclusively to necrotic non-sporulating infections. Stirling Castle was resistant to three of the latter and completely susceptible to the fourth. Strains characterized by the production of non-sporulating infections of a susceptible type on Potentate and similar infections of a resistant type on Stirling Castle are designated as physiologic Form 3 (Plate I, Fig. 3), whereas those producing similar infections of a susceptible type on both of these varieties are designated as physiologic Form 4 (Plate I, Fig. 4).

The similarity of the reactions produced by the mutant Forms 3 and 4 to those caused by normal strains under conditions of low relative humidity was strikingly demonstrated in a midsummer inoculation of six varieties with six cultures of single-spore origin. In all cases the infections were similar and without sporulation. That two types of reaction were involved, however, became evident when the plants were transferred to an inoculation chamber in which the relative humidity was maintained close to saturation, since in four of the six cases the fungus subsequently sporulated profusely while in the remaining cases it failed to sporulate.

The results of these pathogenicity tests have been supported by extensive cultural studies which indicate that the species *C. fulvum* is composed of an indefinite number of physiologic forms. Comparative studies of cultures obtained by isolating single one-celled conidia directly from sporulating infections have shown, on a single medium, differences in such characteristics as growth form and color. Also, as is shown below, many culturally distinct mutant strains have been isolated from a relatively small number of original cultures. It is not claimed that all mutant strains give rise to infections of the type produced by Forms 3 and 4. Furthermore, although the pathogenicity of a large number of original cultures has been tested it is quite possible that here, too, the existence of other physiologic forms may be demonstrated. The cultures used in this investigation were isolated from a comparatively

* Any culture of the fungus which, under conditions of relatively high humidity such as have prevailed in our greenhouses in the spring and autumn of the year, produces sporulating infections on susceptible varieties is designated as a normal strain.

small area. Cultures from other localities may possess different parasitic capabilities and new differential hosts may be found. It seems likely, however, that the immunity of the Red Currant tomato, *L. pimpinellifolium*, will prove operative against all physiologic forms of the fungus, since, in the present investigation, it has shown itself immune from numerous cultures of the fungus and since other workers in widely separated centres (1, 4, 9, 18) have reported it immune from *C. fulvum*.

VARIABILITY OF *C. fulvum* IN CULTURE

Saltations occur frequently in cultures of *C. fulvum*, and the saltant strains, when isolated, exhibit great diversity in such cultural characteristics as growth rate, topography, color, amount of sporulation and the production of watery beads on the surfaces of the colonies.

Some idea of the variability of *Cladosporium* in culture may be obtained by following the behavior of the subcultures from a single spore, No. 51, which was isolated in August 1933, from a two-weeks-old culture from a natural infection. This spore produced on potato-dextrose agar a colony which, after a week's growth, was olive in color and of an irregular mounded contour. After 15 days a suspension of conidia from this colony was used in making cultures in Erlenmeyer flasks. In 11 days the surface of the agar in these flasks was covered with crowded colonies which had fruited abundantly, and at various places on their surfaces loose tufts of light colored mycelium had appeared. In March 1934, subcultures were made to 2% malt agar from one of these flask cultures and there resulted not only olive-colored colonies but also variants, two of which, one buff and one white, were isolated and found to be distinct in growth rate and colonial form as well as in color (Plate II, Fig. 1). The white and buff strains remained constant through five additional transfers extending to February 1935, whereas, from the olive strain during the same period, light-colored variants arose occasionally. The stability of the white and buff strains was further demonstrated in March 1935, when the three strains were passed through the host and recovered unaltered. Subsequently the light-colored strains have retained the same characteristics through five cultural generations on potato-dextrose agar. Two other variants from the olive strain from Spore 51 have been studied for more than a year; both have produced further variants. In February 1936, several saltants differing from any previously isolated were obtained from the same olive strain.

To discover whether the variability of cultures of *C. fulvum* was associated with a multinucleate condition of the conidia they were stained with gentian violet. The conidia were from one- to four-celled, with a single nucleus in each cell (Plate II, Fig. 5). It was evident, then, that the variability associated with a single nucleus could be determined from the study of the variability of cultures derived from single-celled spores. Accordingly, 20 single spores, 15 of them single-celled, were isolated directly from each of six sporulating infections. The original cultures resulting from the growth of these 120 spores showed a striking variability, patches of loose light-colored

mycelium appearing irregularly on practically every culture. The cultures from single-celled conidia were as variable as those from conidia having two cells. While the 20 individuals from each group were very similar in the types and frequency of saltations, differences in these respects were observed from group to group over a period of four months.

Of the original cultures 22 were selected for further study. When these cultures were five weeks old three subcultures were made from the olive-colored portion of each of them. After three months at least two of each group of three subcultures had produced variants. Attempts were made to make isolations from all the variants observed in these three-months-old subcultures, a small weft of mycelium being picked off with a fine needle. Within two weeks these isolates exhibited marked differences in growth rate, production of watery beads, colonial form, and color, including whites, grays, buffs and olive greens. Triplicate cultures of the saltant strains were studied on a single medium and behaved uniformly. As many as seven distinct saltant strains were isolated from cultures resulting from a one-celled, uninucleate conidium.

The evidence presented above indicates that the variability of *C. fulvum* in culture is due to nuclear changes which are considered to be mutations, in the broad sense of the term, but whether they are due to gene mutations or chromosome aberrations cannot be stated.

THE PLASTICITY OF REACTION BETWEEN PURE LINES OF HOST AND PARASITE

In addition to those variations in the reaction of the tomato to *C. fulvum* which are due to the parasitic capabilities of genetically different strains of the parasite, and to those variations which are conditioned by genetic differences on the part of the host, and which will be treated in the section on the inheritance of resistance to *C. fulvum*, variations were observed when a pure line of the host was infected by a pure line of the parasite under various conditions. The influences on reaction type of environmental factors, of developmental stages, and of grafting will be considered.

Environmental Factors

The reaction of a tomato variety to *C. fulvum* has been shown repeatedly to depend on the general vigor of the inoculated plant. On vigorous plants of susceptible varieties little or no chlorosis is evident and the development of the fungus is vigorous, with abundant sporulation. On chlorotic plants and on plants whose growth has been appreciably retarded, infections cause a rapid chlorosis of the infected area, the distribution of the fungus is restricted and sporulation greatly reduced. No analysis has been made of the factors responsible for these variations. Schaffnit and Volk (16) and Volk (21), however, have demonstrated the dependence on nutrition, soil moisture, air moisture and other environmental factors of the expression of the leaf mold disease on completely susceptible varieties of tomato.

The reaction of the Red Currant tomato, *L. pimpinellifolium*, has likewise shown variations. It has been included in at least 100 inoculations carried out during every month of the year. In the vast majority of cases it has not shown macroscopic symptoms after inoculation. However, on five occasions, twice in May 1934, and in September 1934, October 1935, and November 1935, definite macroscopic evidence of infection has been observed on plants that were from four to five weeks old when inoculated. On November 6, 1935, eight days after inoculation with a strain which previously and subsequently failed repeatedly to induce symptoms on plants grown from the same seed lot, Red Currant plants showed minute brown necrotic flecks on the inoculated leaves. Bits of leaf tissue including these were surface-sterilized with 1:1000 mercuric chloride in water for from 1 to 1.5 minutes and plated out. *C. fulvum* was isolated in four out of eight cases, whereas four checks, bits of apparently uninfected portions of the inoculated leaves, failed to yield the fungus. The flecks did not increase in size and an attempt to isolate the fungus from them 21 days after inoculation failed. In May 1934 *C. fulvum* was isolated from similar infections on Red Currant 14 days after inoculation. In at least three other instances symptoms similar to the above have been observed in the F_2 from Red Currant \times esculentum, among individuals bearing the genetic factor for immunity that is found in Red Currant. It cannot be stated what environmental factors are responsible for the development of these symptoms on genetically immune individuals nor has it been possible to cause the production of such symptoms on other occasions.

Atypical symptoms of the disease have been observed also on 2R, a selection from the F_2 of the cross, Tangerine \times Red Currant; 2R has been tested repeatedly and is partially resistant to all known forms of the fungus, the lesions ordinarily failing to sporulate even after a long period of development (Plate III, Fig. 3). In September, 1934, however, infections produced on it by several strains of the fungus were sporulating moderately 15 days after inoculation. As in Red Currant the same strains produced typical infections on plants grown from the same seed lot when inoculations were made at a later date.

Temperature and humidity have been shown by Small (19) to exert a great influence on the development of various stages of the leaf mold disease. Thus, abundant sporulation was observed at 92% and 78% relative humidity, whereas at 58% the disease spots dried out and produced very few spores. The present investigation also showed this dependence of sporulation on relative humidity. When the relative humidity is low, typical sporulating infections are not produced on susceptible varieties by normal strains of the fungus; instead, the invaded leaf tissues are killed rapidly and a necrotic spot with a slowly advancing chlorotic margin results (Plate II, Fig. 4). All possible intergrades between infections of this type and the typical susceptible reaction have been observed. On various occasions plants having such necrotic non-sporulating lesions have been transferred to a large cloth inoculation chamber in which the relative humidity approached saturation.

In all these cases the fungus developed profusely at the margins of the lesions and sporulated within 24 hours. The type of development of infections in the inoculation chamber was the same as that which occurs initially on plants infected under humid conditions. Because of the rapidity with which normal development and sporulation was induced in these cases it is considered that the influence of relative humidity is largely upon the parasite.

The most striking instance of the plasticity of reaction type is the seasonal variation in the reaction of Stirling Castle to Form 1. In midwinter, instead of producing the resistant reaction (Plate I, Fig. 1, B) which has been described above, Stirling Castle reacts like susceptible varieties such as Potentate (Plate II, Fig. 4), except that the resulting spots are smaller (Plate II, Fig. 3).

A gradual change to the winter reaction and a gradual return, in the spring, to the summer reaction were indicated from the results of frequent inoculations carried out at Toronto over a period extending from October 1934 to April 1935. Since during this time length of day and light intensity fell to a minimum and subsequently rose again it seemed possible that light might be partly responsible for the observed variations in reaction. This was further suggested by preliminary experiments during the summer of 1935 in which the summer reaction was shifted slightly towards the winter type when the light experience of the plants had been reduced by covering them daily from noon until dusk with an aerated, black broadcloth cage (Plate II, Fig. 2).

The influence of light was studied in greater detail from weekly inoculations carried out at Toronto beginning October 8, 1935, at greenhouse temperatures well within the range of those enabling the development of reactions of a resistant type during the summer. Each week five- to seven-weeks-old plants of Potentate and Stirling Castle were inoculated with physiologic Form 1. With the approach of winter, in addition to the change in the light, the relative humidity of the greenhouse decreased and this induced a progressive decrease in sporulation. Thus, on Potentate, even the infections resulting from the first inoculation sporulated less than under conditions of high relative humidity. This decrease in sporulation was progressive until, with the inoculation of November 13, sporulation ceased. The influence of light, on the other hand, was evidenced by the amount of hypersensitiveness of the host.

On Stirling Castle, symptoms of essentially the normal summer type resulted from the inoculation of five-weeks-old plants on October 8. In successive inoculations there was a progressive but somewhat fluctuating increase in the size of the infection spots on Stirling Castle until, as a result of the inoculation of November 13, infections of the winter type described above were observed. At this time normal sporulation was observed on plants of both varieties that were covered each night by bell jars to increase the relative humidity.

Commencing December 5 the humidity of the whole greenhouse was greatly increased by placing on the heating pipes large pieces of burlap leading to water reservoirs. A relative humidity of 60%, as recorded on one greenhouse bench by a Julien P. Friez hygrothermograph, was maintained at temperatures

ranging from 70 to 80° F. After inoculation on December 18, symptoms became evident about the twelfth day and were very similar on the two varieties, the spots being diffuse, with a sparse mycelial growth on the under surface of the leaves. The restriction of development of the fungus which is observed on Stirling Castle plants at a comparable stage during the summer months was entirely lacking, and sporulation occurred on both varieties. After 24 days the heavily infected leaves on both varieties were almost completely killed and shrivelled, the necrosis of the infected tissues of Stirling Castle presenting a striking contrast to the chlorosis and lack of necrosis characterizing summer infections under comparable conditions.

On the basis of the results presented above it is considered that the seasonal variation in the reaction of Stirling Castle to physiologic Form 1, under the described range of environmental conditions, is attributable to a great extent to the effects of two factors, light and relative humidity. The reduced light experience of plants grown in midwinter is held to be largely responsible for the lack of expression of the genetic factor for resistance, while low relative humidity has been shown to be predominantly responsible for the lack of sporulation.

Developmental Stages

In the great majority of more than 150 inoculations, plants from one to two months old have been used and it has been observed repeatedly that symptoms appeared and developed later on the upper younger leaves than on the lower more mature ones. A regular gradation in time was observed but the type of reaction did not differ otherwise. On older plants with firm senile lower leaves a second gradation has often been observed. On the central and upper leaves of such Potentate plants normal infections developed, whereas the lower leaves displayed a progressive reduction in sporulation, the lowest showing only diffuse grayish spots which failed to sporulate even after a long period of development. The reaction of a leaf will thus be determined by its physiologic age.

Grafting

The reaction to *C. fulvum* of the components of grafts between *L. esculentum* and various solanaceous plants has been studied in considerable detail by Volk (21) and Bond (4). Bond has also studied the influence of grafting on the reaction of components of grafts between esculentum varieties known to differ in their reaction to *C. fulvum*. In the components of three graft combinations he found no change in reaction type due to grafting. Similar observations have been made during the present investigation. Following the inoculation of grafts between the esculentum varieties Potentate (completely susceptible) and Stirling Castle (resistant to physiologic Form 1), Potentate being used both as stock and as scion, no departure from the normal reactions for these varieties was observed.

STUDIES OF THE INHERITANCE OF RESISTANCE TO *C. fulvum**Host Range and Varietal Reaction*

Pathogenicity tests have been restricted to species of the genus *Lycopersicum*, and to *Solanum melongena*, the only other species reported susceptible to this fungus (7). Young plants of the eggplant varieties Black Beauty and Blackie were found to be immune from strains of the fungus that produced typical symptoms of leaf mold on susceptible varieties of tomato inoculated at the same time. Tests have been made of the reaction of 51 *L. esculentum* varieties which included both old and modern commercial types as well as small-fruited varieties; of *L. Humboldtii* from two sources; of *L. pimpinellifolium* from several sources; of hybrids between *L. esculentum* and *L. pimpinellifolium*; and of two wild tomatoes whose relationships to these species are uncertain. Four distinct types of reaction to normal strains of *C. fulvum*, each of which may be modified by environmental factors or by genetic factors in the host, were revealed by these tests:

1. *Potentate*. Potentate is completely susceptible to all known physiologic forms of the fungus. On it are produced the normal symptoms of the disease (Plate I, Fig. 1, A; Plate III, Fig. 1). Other completely susceptible varieties are: *L. esculentum* varieties Acme, Ailsa Craig, Ailsa Craig green stem mutant a_2 , Banalbufar, Beauty, Bonny Best, Break O'Day, Buckeye State, Burbank Preserving, Chalk's Early Jewel, Cooper's Special, Crackerjack, Denton's Special, Devon Surprise, Dwarf Aristocrat, Dwarf Stone, Earliana, Early Detroit Purple, First of All, Golden Queen, Golden Dwarf Champion, Grand Rapids, Grape Cluster, Honor Bright, Imperial Globe, King Humbert, Marglobe, McMullen Pink, Norton, Oxheart, Peach, Ponderosa, Pritchard, Red Cherry green stem mutant a_1 , Red Pear, Rouge Naine Hative, Rough Trophy, Satisfaction, Stone, Sunrise, Tangerine, Wonder of Italy, Yellow Pear, Yellow Plum and five selections made by J. W. MacArthur; selections of *L. Humboldtii* from Copenhagen and Frankfurt; a hybrid received as *L. pimpinellifolium* fructo luteo and wild tomatoes from Jamaica and Mexico.

2. *Stirling Castle*. This variety is completely susceptible to physiologic Forms 2 and 4 but resistant to Forms 1 and 3. The Stirling Castle type of reaction (p. 110; Plate I; Plate III, Fig. 2) occurred in the *esculentum* varieties Best of All, Maincrop, Norton's Wilt-resistant Stone (not homozygous), Tuckswood Favourite and Up-to-date.

3. *2R selection*. This selection (p. 114) is resistant to all known forms of the fungus. Under conditions of abundant light and relatively high humidity, symptoms appear in about the same time as on Potentate and Stirling Castle but unless the relative humidity is very high the fungus does not grow out through the stomata to an appreciable extent, even after a long period of development. The infected tissue becomes chlorotic and the resulting spots are similar in appearance on both surfaces of the leaf (Plate III, Fig. 3). Necrosis is not evident by the time infections on comparable Potentate plants are sporulating profusely, but usually some necrosis occurs eventually, its

extent varying from plant to plant. The single instance in which sporulating infections were observed on 2R has been described above. Three sixteenths of the F_2 individuals of crosses between *L. pimpinellifolium* and completely susceptible varieties react similarly to 2R.

4. *Red Currant*. Although this species of tomato is ordinarily considered immune from *C. fulvum* and macroscopic symptoms do not typically follow inoculation (Plate III, Fig. 4), bits of leaf tissue cleared and stained in lactophenol with cotton blue show that the fungus penetrates the plant through the stomata and develops in the leaf tissue to a very limited extent. Furthermore, in five instances among more than 100 inoculations infection flecks have been observed on Red Currant (p. 114). The immunity reaction was observed only in *L. pimpinellifolium* and in derivatives from it.

Mendelian Nature of the Factors Governing Resistance

The Two Resistance Factors of the Red Currant Tomato

Tests of entire F_2 populations of crosses between the immune Red Currant tomato and typically susceptible esculentum varieties have been carried out to discover whether a linkage exists between the factor for immunity and some factor or factors included in MacArthur's (12) chromosome maps. In addition to the parental types a third class of individuals, partially resistant to all known forms of the fungus, was observed in such populations. Among the latter there was considerable variation, even under the same environmental conditions, and it was often difficult to classify the individuals at the extremes of this range. Under conditions of relative humidity enabling the development of sporulating infections on Potentate the majority produced non-sporulating infection spots similar to those described for 2R selection. Under very humid conditions some individuals sporulated but they could be distinguished from typically susceptible individuals on which the spots spread over a greater area. Resistant individuals of this type have been found in all F_2 populations of Red Currant crosses but never among esculentum varieties.

The genetic basis of these results is elucidated by the data secured from the inoculation, in the summer of 1934, of 500 F_2 individuals of a cross, Red Currant \times esculentum. Because of the wide range of symptoms resulting it was necessary to inoculate some of the plants several times before a conclusive classification could be made. The final observed ratio was 379 immune : 90 partially resistant : 31 typically susceptible. This conforms very closely to a 12 : 3 : 1 ratio which is 375 : 93.8 : 31.2 for a population of 500. The results secured in this instance have been supported by those obtained from all other F_2 populations. They are explained, on the basis that *L. pimpinellifolium* carries, in addition to the dominant factor for immunity, an independently segregating dominant factor for resistance which is expressed only in the absence of the factor for immunity, *i.e.*, is hypostatic to it. The symbol Cf_{p1} is assigned to the factor governing immunity and the symbol Cf_{p2} to the resistance factor. The single factor basis of these

two resistances and the dominance of the two factors are confirmed by back-cross data. For instance, the inoculation of 178 plants from three populations yielded the ratio 97 immune : 44 resistant : 37 susceptible; the deviations from theoretical 1 : 1 ratios are 1.2 and 0.7 times the standard error for Cf_{p1} and Cf_{p2} , respectively.

The factor Cf_{p1} behaves as a complete dominant, even in the presence of a chromosome complex derived largely from typically susceptible esculentum varieties. On the other hand, there is considerable evidence that the Cf_{p2} factor is not fully dominant. In some inoculations it seemed possible to divide the resistant individuals into heterozygotes and homozygotes; the suspected homozygotes displayed infections resembling those on 2R selection, which is known to be homozygous for Cf_{p2} , and the suspected heterozygotes showed larger infections with necrosis or some sporulation. However, this possibility was not tested by the growing of F_3 populations.

Conspicuous among the factors modifying the expression of the main resistance factors is the lutescence factor which in the homozygous condition not only determines lutescence but also modifies the expression of the Cf_{p1} factor. On lutescent, genetically immune individuals from crosses between Red Currant and esculentum varieties there appear, following inoculation, small conspicuous chlorotic spots the centres of which rapidly become necrotic. These spots are much more conspicuous than the flecks that have been observed a few times on non-lutescent, genetically immune individuals, but like them do not increase noticeably in size, their maximum diameter being less than 2 mm. (Plate II, Fig. 6). The contrasting reaction of a partially resistant, lutescent hybrid is shown in Plate II, Fig. 7.

The Resistance Factor of Stirling Castle

The variety Stirling Castle has long been recognized as somewhat resistant to *C. fulvum* (11). This resistance has been described and it has been noted that Stirling Castle is resistant to Forms 1 and 3 but completely susceptible to Forms 2 and 4. While the following results are based on reactions to Form 1 it is believed that similar results would be obtained with the closely related Form 3.

The reaction of F_1 individuals from crosses between Stirling Castle and seven completely susceptible varieties (Burbank Preserving, Potentate, Rouge Naine Hative, a wild tomato from Mexico and three esculentum selections) was tested on several occasions during the summer months. In all cases their reaction was very nearly the same as that of comparable Stirling Castle plants, thus demonstrating that under the prevailing environmental conditions the resistance of Stirling Castle is dominant.

From nine inoculations of a total of 773 F_2 individuals from five such crosses the observed ratio was 557 resistant : 216 susceptible, which deviates from a 3 : 1 ratio by 1.8 times the standard error. The resistance of Stirling Castle, therefore, is governed by a single dominant Mendelian factor. The symbol Cf_{sc} is assigned to this factor, the distinctness of which from Cf_{p2} is evident

from the fact that the latter confers resistance to all known forms of the fungus, whereas Cf_{sc} does not confer a resistance to Forms 2 and 4, and also from the fact that an F_2 of the cross, 2R selection \times Stirling Castle, yielded completely susceptible individuals as well as parental types. The degree of dominance of the Cf_{sc} factor has been shown to vary with varying environmental conditions. In a number of the inoculations the resistant and susceptible reactions were so clearly defined that there could be no possible doubt as to the classification of an individual. In June 1935, however, amongst a population of 215 F_2 individuals, a wide range of reactions was observed, extending from typical resistant to typical susceptible types. The amount of sporulation ranged from profuse on typically susceptible individuals, through very slight on individuals which certainly carried one Cf_{sc} factor, to none at all on other individuals. A few individuals could not with certainty be classified as resistant or susceptible. The fact that more than two types of reaction were observed demonstrates the incompleteness of the dominance of the resistance factor under the conditions of the experiment, whereas the wide range of reactions among genetically resistant individuals indicates the presence of modifying factors. These variations are probably attributable in large measure to very high relative humidity and to the extremely rapid growth of the plants. This explanation seems more likely in view of the fact that a clearly defined segregation of resistant and susceptible individuals resulted from the inoculation, five days later, of smaller less vigorous plants of the same F_2 population. It is also pointed out that under conditions enabling the expression of Cf_{sc} as a nearly complete dominant the effect of modifying factors is scarcely apparent, the range of symptoms being very narrow.

When backcross populations of Stirling Castle crosses were inoculated in June and July 1935, a sharp segregation of resistant and susceptible individuals resulted. The observed ratio of 194 resistant : 179 susceptible, which deviates from a 1 : 1 ratio by 0.9 times the standard error, demonstrates further the single factor basis of the resistance of Stirling Castle. In these populations some of the resistant individuals supported slight to moderate sporulation; as all the resistant individuals were heterozygous for Cf_{sc} , this variation in the amount of sporulation is a clear demonstration of the presence of modifiers. In those instances in which Stirling Castle was crossed with a lutescent parent the recessive factor governing lutescence was outstanding as a modifier of reaction type. On completely susceptible individuals lutescence caused a more pronounced gradation from sporulating to non-sporulating infections than has been observed on plants with senile leaves. In many cases this gradation was pronounced, even on single leaves intermediate in position between leaves with and without sporulation; here, terminal leaflets showed no sporulation while infections on the younger lateral leaflets were sporulating profusely. On both resistant and susceptible individuals lutescence causes a shortening of the incubation period of *C. fulvum*, symptoms regularly appearing from one to two days earlier than on normal non-lutescent individuals. In addition,

the infections on lutescent individuals are of a bright yellow color and are strikingly conspicuous on those upper leaves which are still green. A separation of hybrid populations into lutescent and normal individuals can be made readily on the basis of these differential reactions.

Linkage Relations of the Factors Governing Resistance

Linkage tests applied to the three resistance factors involved 19 pairs of characters whose monofactorial inheritance has been proved by MacArthur (12) and Butler (5). Data were secured from a study of F_2 and of backcross populations of crosses made in the coupling phase, *i.e.*, both dominants entered the cross from one parent and both recessives from the other.

Cf_{p1} , the Factor for Immunity

The linkage relations of this factor with 15 others distributed on nine chromosomes are summarized in Table I. It will be seen that Cf_{p1} is situated

TABLE I

LINKAGE DATA FOR Cf_{p1} , THE FACTOR FOR IMMUNITY, SECURED FROM F_2 POPULATIONS

Factor pair tested	Chromosome number on which located	Segregation ratios*				Number of plants tested	Percentage crossing over with standard error	Deviation in terms of the standard error
		XY	Xy	αY	αy			
$D_1 d_1$	I	893	223	285	71	1472	50.0 \pm 1.9	0.0
$P p$	I	301	77	97	24	499	50.5 \pm 3.4	0.1
$R r$	II	282	97	97	24	500	54.6 \pm 3.1	1.5
$Y y$	III	382	108	122	37	649	49.0 \pm 3.0	0.3
$C c$	IV	787	217	214	98	1316	43.0 \pm 1.9	3.7
$F f$	V	302	77	92	29	500	47.0 \pm 2.8	1.1
$A_1 a_1$	V	529	181	162	64	936	47.8 \pm 2.4	0.9
$L_f l_f$	V	306	73	87	34	500	43.2 \pm 2.8	2.4
$J j$	V	304	75	86	35	500	43.1 \pm 2.8	2.5
$L l$	VI	728	231	228	63	1250	51.5 \pm 2.2	0.7
$U u$	VII	82	29	28	10	149	49.9 \pm 6.1	0.0
$H h$	VII	1374	404	427	144	2349	48.1 \pm 1.5	1.3
$T t$	VII	82	29	28	10	149	49.9 \pm 6.1	0.0
$A_2 a_2$	VIII	125	32	55	10	222	54.8 \pm 5.3	1.0
$Wt wt$	X	323	56	106	15	500	52.8 \pm 3.4	2.1

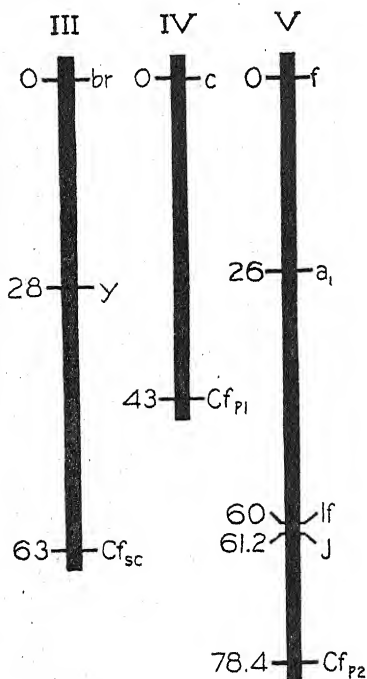
* In this and in subsequent tables:

X = the dominant and x = the recessive of the factor pair governing resistance and susceptibility.

Y = the dominant and y = the recessive of the other factor pair tested.

on chromosome IV, loosely linked with c , the factor for potato leaf (Text-fig. 1). The position of this immunity factor on the fourth chromosome cannot be stated more accurately until an examination is made of its linkage relations with sp , the factor for self-pruning habit of growth, which is very closely linked with c . Tests were also carried out to determine whether there was any linkage between the immunity factor and factors determining fruit size. For this purpose 534 F_2 plants of two Red Currant \times esculentum crosses

were grown to maturity in the field. Mean fruit weights were determined from the averages of 20 ripe fruits per plant and the deviations expressed in terms of the standard error. In one population, comprising 384 plants, the mean fruit weights of Cf_{p1} and cf_{p1} individuals were 7.2 ± 0.1 gm. and 7.3 ± 0.4 gm. respectively and the insignificant difference between the two classes 0.1 ± 0.4 gm. From a second population the corresponding figures were 10.0 ± 0.4 gm. and 10.4 ± 1.0 gm. with a difference of 0.4 ± 1.2 gm., again not a significant one. Although these data show no indication of a linkage between the immunity factor and fruit size factors, an article by MacArthur and Butler (13) indicates that such a linkage might be discovered in populations from other crosses.



TEXT-FIG. 1. Chromosomes III, IV, and V, showing loci of Cf_{sc} , the resistance factor from Stirling Castle, Cf_{p1} , the immunity factor from the Red Currant tomato, and Cf_{p2} , the resistance factor from the Red Currant tomato.

Cf_{p2} , the Resistance Factor from Red Currant

Since the linkage data involving this factor were secured from the same populations that furnished the data for the immunity factor, only about one-fourth as many plants are included in the ratios observed. The linkage relations of the resistance factor are summarized in Table II, from which it is seen that Cf_{p2} is located on chromosome V, linked fairly

TABLE II

LINKAGE DATA FOR Cf_{p2} , THE RESISTANCE FACTOR FROM RED CURRANT, SECURED FROM F_2 POPULATIONS

Factor pair tested	Chromosome number on which located	Segregation ratios				Number of plants tested	Percentage crossing over with standard error	Deviation in terms of the standard error
		XY	Xy	xY	xy			
$D_1 d_1$	I	24	5	13	2	44	54.3 ± 11.8	0.4
$P p$	I	72	18	25	5	121	50.6 ± 6.8	0.0
$R r$	II	73	17	24	7	121	46.8 ± 6.5	0.5
$Y y$	III	94	27	28	10	159	47.0 ± 5.8	0.5
$C c$	IV	22	7	10	5	44	43.7 ± 10.5	0.6
$F f$	V	71	19	21	10	121	42.0 ± 6.2	1.3
$A_1 a_1$	V	24	5	11	4	44	42.3 ± 10.4	0.7
$Lf lf$	V	78	12	9	22	121	18.4 ± 4.0	7.9
$J j$	V	78	12	8	23	121	17.2 ± 3.8	8.6
$U u$	VII	23	8	5	2	38	48.0 ± 11.8	0.2
$H h$	VII	117	33	36	17	203	42.9 ± 4.9	1.4
$T t$	VII	22	9	6	1	38	$>60 \pm 10.4$	1.0

closely with *j* and *lf*, factors for jointless pedicel and leafy inflorescence respectively. The locus of the resistance factor on chromosome V is plotted in Text-fig. 1.

Cf_{sc}, the Resistance Factor from Stirling Castle

Table III summarizes the linkage relations of this factor with 17 others distributed on 10 chromosomes. It is seen that *Cf_{sc}* is linked with the *Yy* factor pair, which governs the character contrasts yellow *vs.* clear fruit epicarp.

TABLE III
LINKAGE DATA FOR *Cf_{sc}*, THE RESISTANCE FACTOR FROM STIRLING CASTLE

Factor pair tested	Chromosome number on which located	Segregation ratios				Number of plants tested	Percentage crossing over with standard error	Deviation in terms of the standard error
		XY	Xy	xY	xy			
F ₂ data:								
D ₁ d ₁	I	135	41	66	23	265	48.1 ± 4.4	0.4
P p	I	135	40	64	24	263	49.9 ± 4.6	0.0
O o	I	115	60	56	32	263	48.7 ± 4.6	0.3
S s	I	135	40	68	20	263	51.1 ± 4.7	0.2
R r	II	137	38	70	16	261	51.7 ± 4.7	0.4
Y y	III	148	27	48	38	261	30.9 ± 3.6	5.3
Backcross data:								
D ₁ d ₁	I	90	104	99	80	373	54.4 ± 2.5	1.8
R r	II	59	42	34	52	187	40.7 ± 3.6	2.6
Br br	III	45	48	46	47	186	50.5 ± 3.7	0.1
Y y	III	61	40	35	51	187	40.1 ± 3.6	2.8
C c	IV	59	42	45	41	187	46.5 ± 3.7	0.9
F f	V	50	43	53	39	185	51.9 ± 3.7	2.2
A ₁ a ₁	V	56	45	48	38	187	49.7 ± 3.7	0.1
L _f l _f	V	36	57	42	50	185	53.5 ± 3.7	0.9
J j	V	36	57	43	49	185	54.1 ± 3.7	1.6
L l	VI	54	47	34	52	187	42.3 ± 3.6	2.1
H h	VII	50	43	50	42	185	50.3 ± 3.7	0.1
A ₂ a ₂	VIII	47	46	42	50	185	47.6 ± 3.7	0.6
D ₂ d ₂	IX	46	47	45	47	185	49.7 ± 3.7	0.1
Wt wt	X	53	40	42	50	185	44.3 ± 3.7	1.5

Whereas the backcross gives a crossover value between these factors of 40.1%, the *F₂* gives the value 30.9%. Since these two measures are equally significant the true crossover value between *Cf_{sc}* and *y* is approximately 35% (Text-fig. 1).

Discussion

Variant strains of *C. fulvum* have heretofore been reported only by Caldis and Coons (6), who considered that they were Dauermodifikationen. They did not, however, present acceptable evidence of their reversion to the parental form, nor have the present studies of the variability of *C. fulvum* revealed a single instance of even apparent reversion. Moreover, variants have appeared

suddenly, at irregular intervals and in localized positions haphazardly disposed, and although many differ but slightly from parental forms, other variants are so distinct that they might not be recognized as *C. fulvum*. In view of these facts and since many distinct variants may be isolated from the product of growth from a single-celled uninucleate conidium it is considered that they arise as a result of mutations, in the broad sense of the term outlined by Dickinson (8).

Since *C. fulvum* is propagated asexually, no perfect stage having been reported throughout its wide geographic range, it is interesting to speculate as to what extent the variability of the fungus in culture is characteristic of its behavior in nature. The existence of a number of physiologic forms in nature has been indicated by pathogenicity tests and by observations of original cultures and, unless we assume the extreme position that the constitution of the species has been static for many years, the most reasonable explanation of the occurrence of physiologic forms is that they arise as a result of mutations.

Only one light-colored form has been isolated directly from nature in about 300 attempts, whereas such forms compose a large percentage of the mutants isolated from artificial cultures. This may be due in part to the failure of some of these forms to persist, inasmuch as inhibition or reduction of sporulation has characterized many light-colored mutants. Another possible explanation arises from our pathogenicity tests which, though limited in number, indicate that many mutant strains, including some which sporulate freely in culture, are unable to sporulate on the host. On the other hand it is conceivable that the stimuli responsible for their frequent occurrence in culture do not operate to the same extent in nature.

Whether the mutations in *C. fulvum* are chromosomal aberrations or gene mutations or whether both these types of hereditary changes occur, cannot be stated. The possibility of arriving at a decision on this question from a study of hybrids is precluded by the absence of a perfect stage. *C. fulvum* possesses, however, many qualities which make it valuable for a study of variation. Pure lines may be obtained readily, the growth of the fungus in culture is relatively slow, mutations occur frequently and many of them are striking in cultural characteristics. Furthermore, distinct qualitative differences in the symptoms of disease are observed and some mutants show changes in pathogenicity. Thus, it is considered that further studies of the variability of *C. fulvum* may add materially to our knowledge of the mechanisms of variation in the Fungi Imperfecti.

The reaction of the tomato to *C. fulvum* is very plastic. Schaffnit and Volk (16), Volk (21) and Small (19) have carried out careful experiments that demonstrate the dependence of the reaction of completely susceptible varieties on various environmental factors. There are, however, no reports in the literature concerning the modification of the reaction of resistant varieties. In the present investigation it has not been possible to relate the rare and irregular occurrence of symptoms of infection on the typically immune

Red Currant tomato to the effect of specific environmental factors. On the other hand, the seasonal variation in the reaction of the variety Stirling Castle to physiologic Form 1 under our experimental conditions has been shown to be due in large part to the influences of relative humidity and light. The demonstration that the failure of the fungus to sporulate on diseased plants during the winter months is due to low relative humidity is in accord with the results of Small (19) and Volk (21). Although a definite relation between reduction of the light experience of plants and the modification of their reaction in the direction of susceptibility has been revealed, our experiments are to be regarded as preliminary ones. Such factors as soil type, nutrition, soil moisture and age of plants were not well controlled and from the work of Schaffnit and Volk (16) and Volk (21) it is apparent that these and other factors must be controlled if uniform experimental material is to be available. Our results do not suggest the manner in which light is operative, but the solution of this striking instance of the dependence of the expression of a known genetic factor on environmental conditions would be a valuable contribution to our knowledge of the nature of disease resistance.

The necessity of employing, when possible, distinct qualitative differences in host reaction for the estimation of resistance and susceptibility to fungi has been illustrated during the course of the present investigation. Von Sengbusch and Loschakowa-Hasenbusch (18) failed to make a complete separation of types of reaction to *C. fulvum* in their study of F_2 populations of the general cross, Red Currant \times completely susceptible esculentum varieties, and thus reported a segregation ratio of 3 immune : 1 susceptible for such populations. In our tests of similar F_2 populations a ratio of 12 immune : 3 partially resistant : 1 completely susceptible has been observed and thus an additional type of resistance has been revealed.

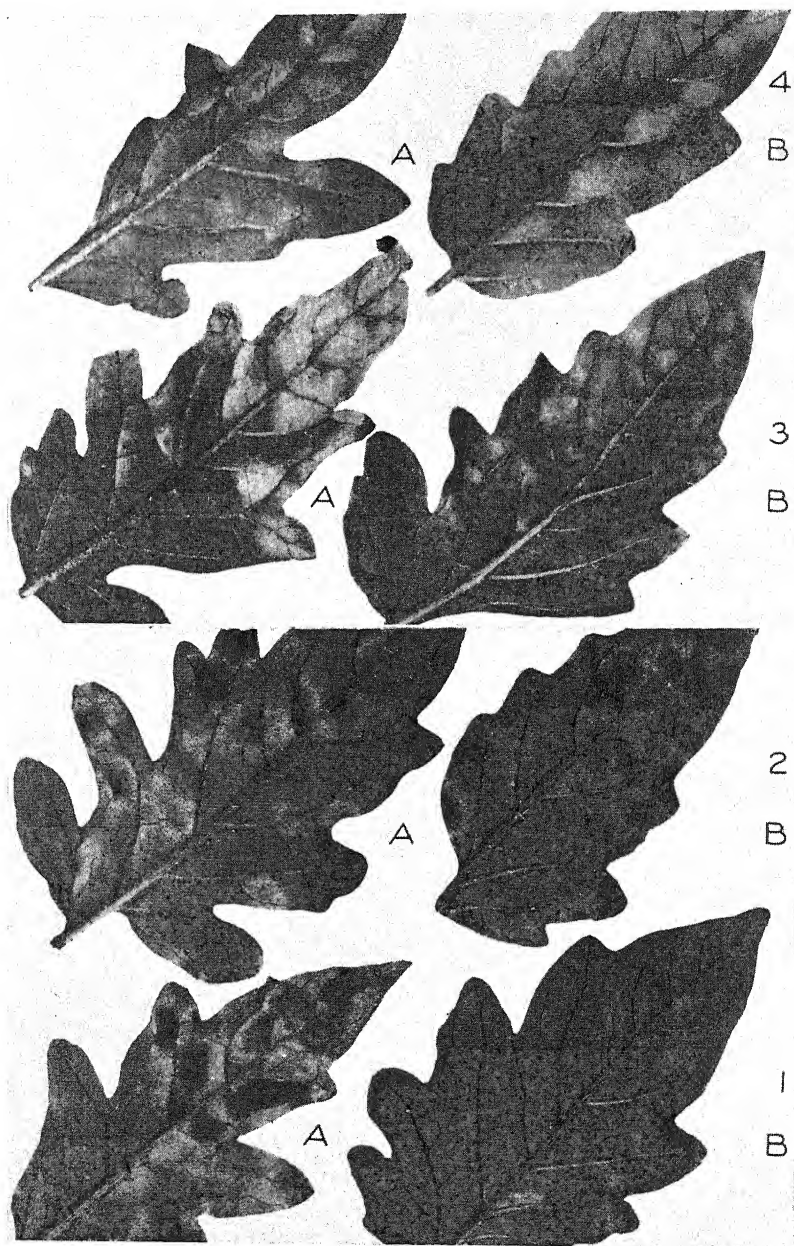
Guba (9) has summarized the conflicting reports of fourteen investigators concerning the occurrence of resistance to *C. fulvum* among the esculentum varieties Frogmore Selected, Lucullus, Norton, Satisfaction, Stirling Castle, Stone, Tuckswood and Up-to-date. There is similar disagreement concerning the inheritance of resistance in these varieties: the resistance of Stirling Castle is reported both dominant (19) and recessive (2, 18), as is that of Satisfaction (2, 15), while resistance is reported recessive in Maincrop (15). Small's F_1 data (19) indicate that the resistance of Up-to-date is dominant, yet a later report (3) states that the F_2 of certain crosses with Up-to-date yielded only susceptible individuals while other crosses with this variety yielded both resistant and susceptible individuals. Our studies of physiologic specialization and of the plasticity of host-parasite relations are significant in relation to the data just given. The variety Stirling Castle is resistant to physiologic Forms 1 and 3 but completely susceptible to Forms 2 and 4. Although Makemson (14), Caldis and Coons (6) and Bond (4) isolated single spores of *C. fulvum*, other workers have used spores from natural infections for inoculation purposes, and it is considered that the failure to relate the results secured on different occasions to specific physiologic forms may explain many of the

contradictions outlined above. In connection with resistance studies a recognition of the plasticity of the reaction between pure lines of host and parasite is equal in importance to that of the existence of physiologic forms of *C. fulvum*. It has been shown that the degree of dominance of the genetic factor governing the resistance of Stirling Castle may vary with varying environmental conditions over a brief space of time and it is suggested that this circumstance may have led to some confusion in inheritance studies. More important, however, are the seasonal variations in the reaction of Stirling Castle, whose inherent resistance is but slightly expressed during midwinter, at Toronto. Varieties resistant during the summer would be classified as susceptible during midwinter. It may readily be seen that an exact assessment cannot be made of the responsibility of each of the factors just considered for the contradictory nature of the available data, but it is clear that these data concerning the resistance of esculentum varieties to *C. fulvum* must be reanalyzed. Although our studies have not revealed any differences among six such resistant varieties it is possible that these may be differentiated through the discovery of other physiologic forms of the fungus. In connection with a reanalysis of the resistance problem it is essential that the reaction of hybrids and of parents be tested simultaneously and that the environmental conditions under which a variety is resistant, as well as the relation of this resistance to physiologic forms of the fungus, be defined clearly.

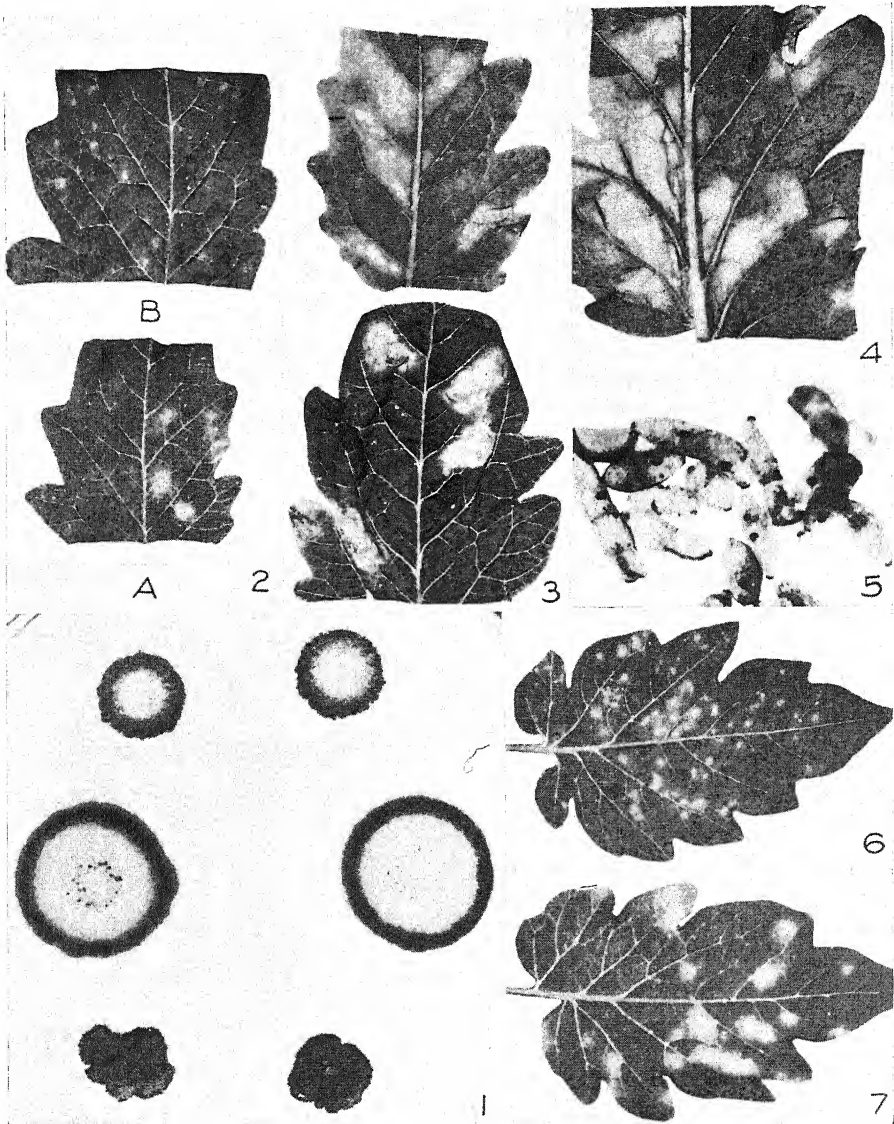
The dependence of the expression of those main factors governing resistance to *C. fulvum* on other genetic factors in the host, as well as on environmental conditions, has been shown clearly. Modifiers of the factors governing the two partial resistances have been demonstrated repeatedly from the behavior of F_2 and of backcross populations. The only modifier that has been recognized apart from its effect on host reaction is that governing the production of lutescent foliage. In contrast to the two resistance factors, the immunity factor of the Red Currant tomato has been singularly independent in its effect, except in the presence of two of the recessive factors governing lutescence. This independence of the factor governing immunity is of great practical significance from the standpoint of developing commercially desirable immune varieties. During the last three years lines carrying this factor have been backcrossed repeatedly to completely susceptible commercial varieties of tomatoes and it has been found that, even in the presence of a chromosome complex derived largely from esculentum varieties, the expression of this immunity factor is not modified.

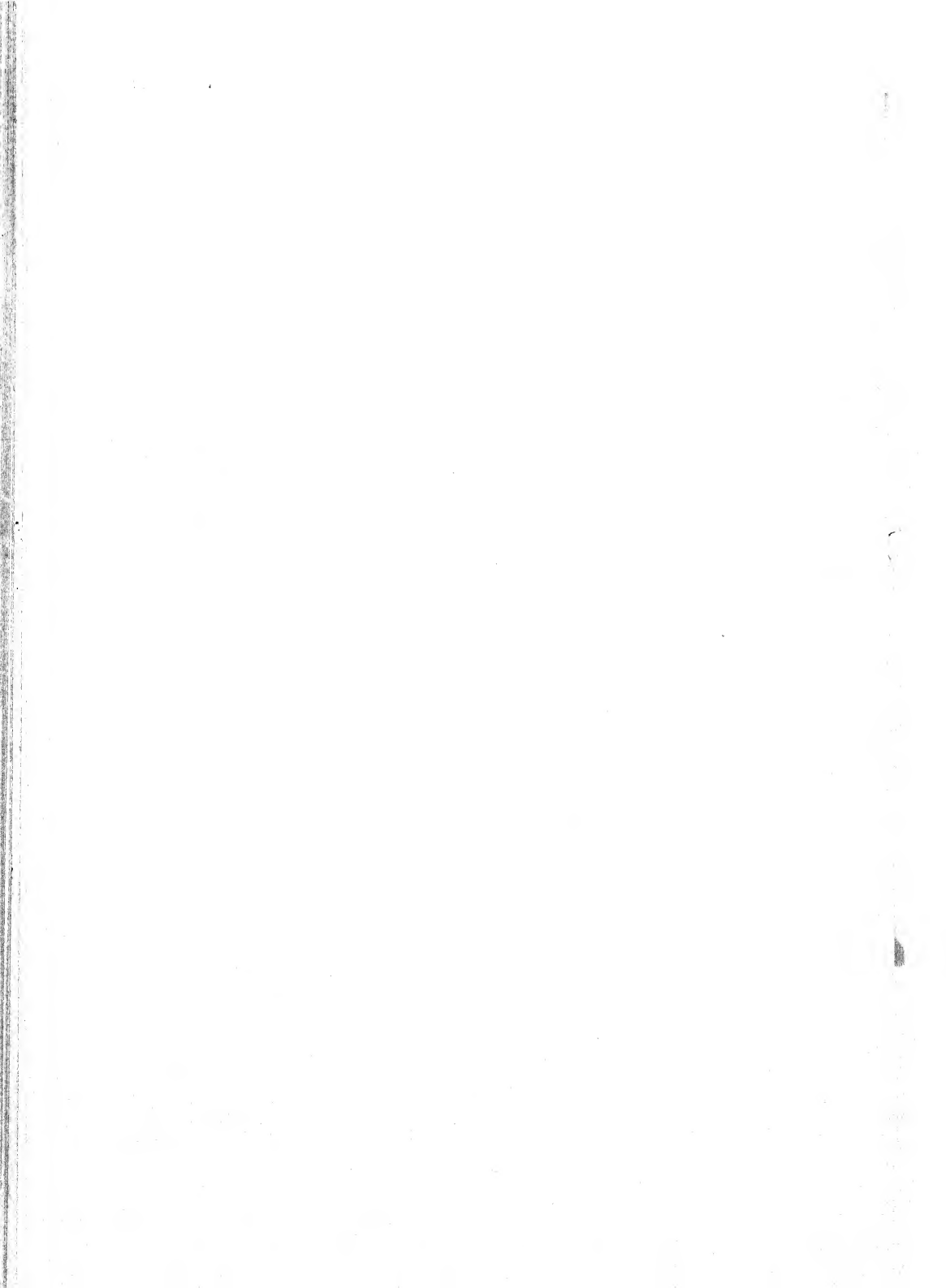
The present studies of varietal reaction and of the inheritance of resistance to *C. fulvum* have involved three types of resistance. The immunity of the Red Currant tomato and the genetic basis of this immunity had been reported already by other workers (18) but the resistance of Stirling Castle has been defined clearly for the first time and a third type of resistance (also found in the Red Currant tomato) has been discovered.

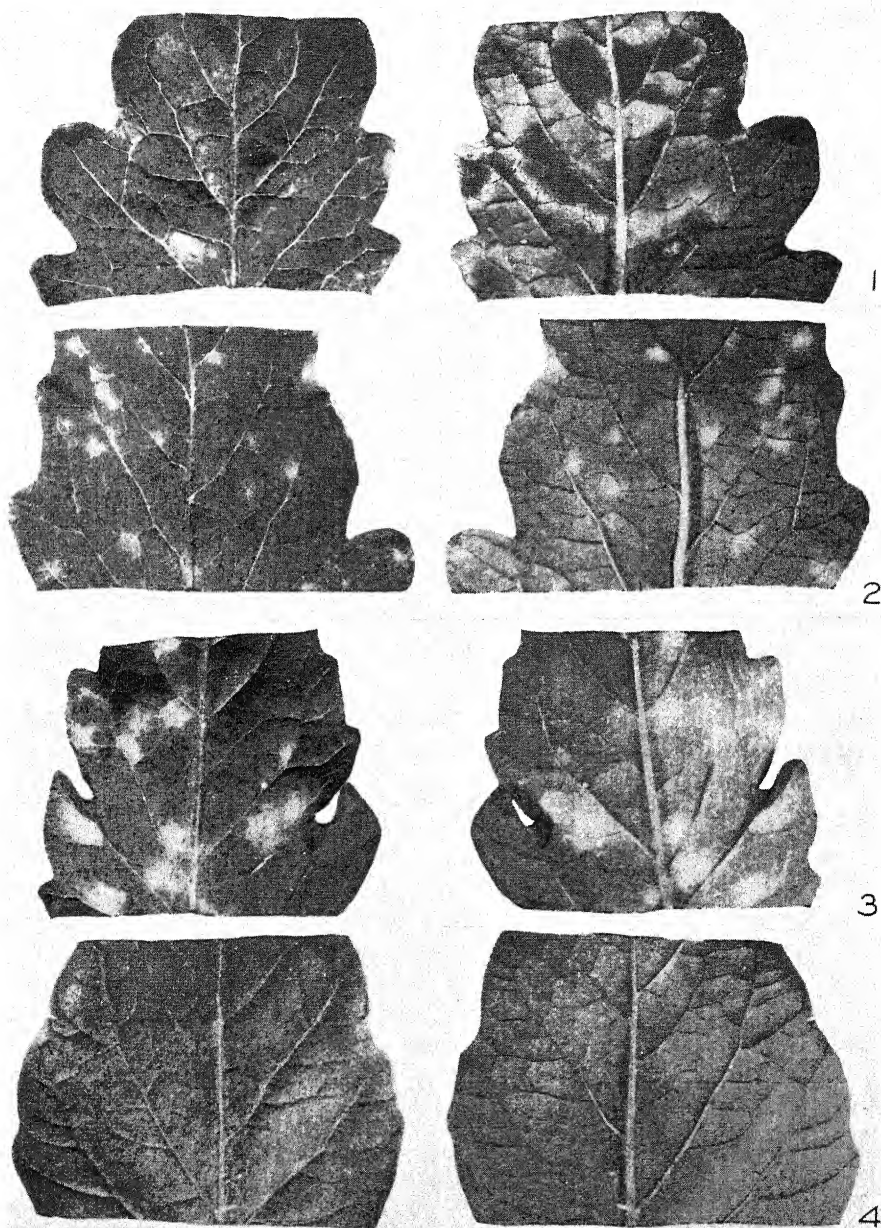
To represent the dominant factors governing these three resistances, universally significant symbols were selected, which would not lead to con-



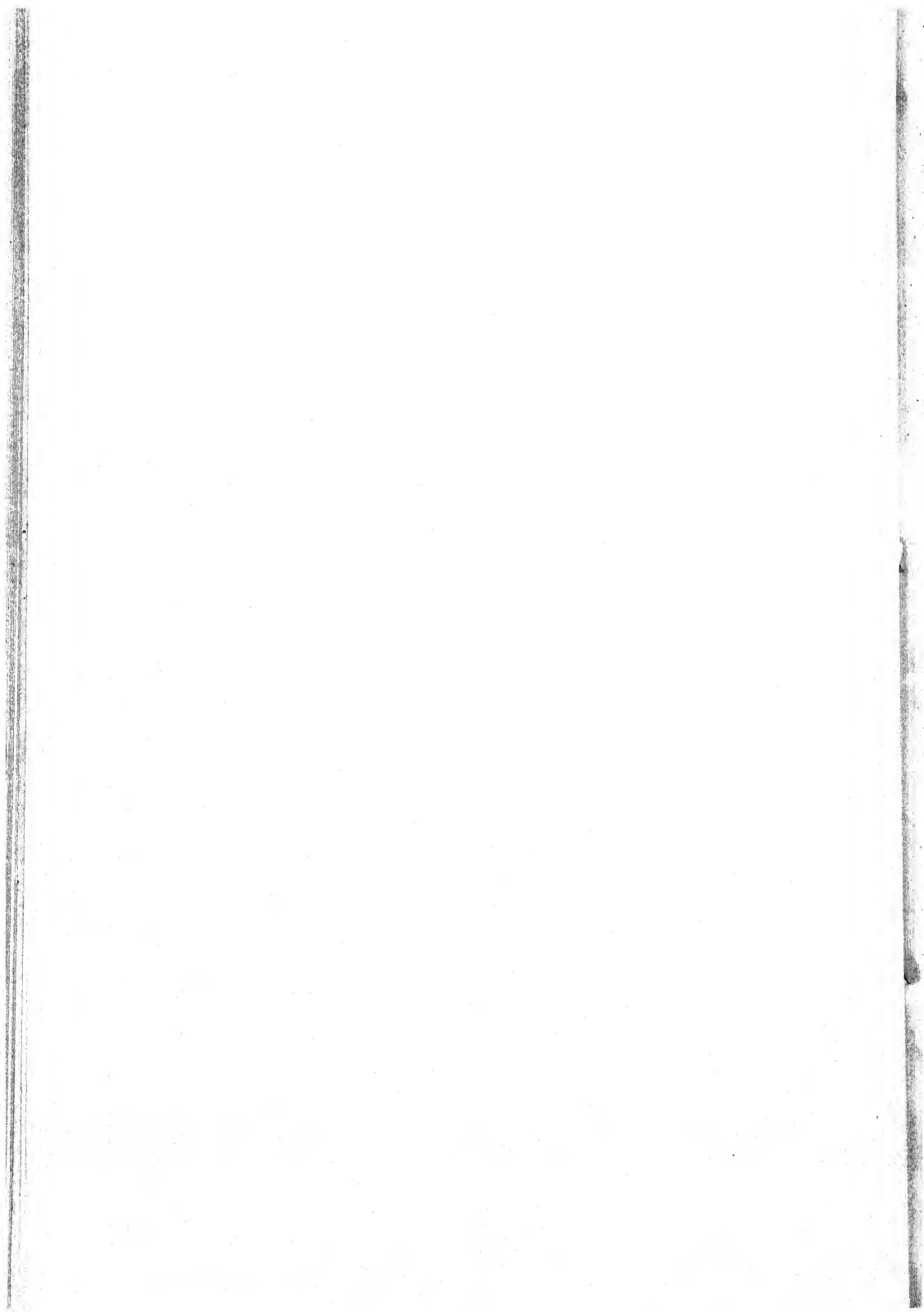
FIGS. 1, 2, 3 AND 4. Reactions of Potentate (A) and Stirling Castle (B) to physiologic Forms 1, 2, 3 and 4, respectively, of *C. fulvum*. Lower leaf surfaces 16 days after the inoculation of 29-days-old plants in September 1934, under conditions of high relative humidity. $\times 1$.







FIGS. 1 TO 4. Reaction of vigorous mature plants of Potentate (Fig. 1), Stirling Castle (Fig. 2.), 2R Selection (Fig. 3.) and Red Currant (Fig. 4.) to *C. fulvum* physiologic Form 1, following a natural infection in August, 1935. Upper and lower leaf surfaces. $\times 1$.



fusion, should further resistance factors for this or other diseases be discovered in the tomato:

Cf_{p1} —for the factor governing immunity.

Cf_{p2} —for the factor governing partial resistance to all known forms of the fungus.

Cf_{sc} —for the factor governing partial resistance to Forms 1 and 3, but not conferring resistance to Forms 2 and 4.

In these symbols Cf signifies a resistance to *C. fulvum* whereas the subscript indicates where the factor was first discovered: thus, p stands for *pimpinellifolium*, the species name of the Red Currant tomato and 1 and 2 signify the first and second resistances discovered in this species; sc refers to the esculentum variety Stirling Castle. It is hoped that other workers will employ this system of symbols if other resistance factors are discovered in the tomato. All three resistance factors have been located in MacArthur's (12) chromosome maps of the tomato. Such precise mapping of specific qualitative factors for disease resistance is probably unique in plants, although Abegg and Owen (1) have reported an instance of linkage between a factor for resistance to curly-top and a factor governing crown color in beets.

Acknowledgments

I wish to express my gratitude for the assistance rendered by Professors D. L. Bailey, and J. W. MacArthur, under whose joint direction this investigation was carried out, and to Professor MacArthur for providing certain genetic materials. My thanks are also due to Professor E. F. Palmer for his cooperation in placing at my disposal the facilities of the Horticultural Experiment Station, Vineland Station, Ontario.

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MICROTECHNIQUE FOR WINTER BUDS¹

BY HUGH P. BELL² AND VERA FACEY³

Abstract

When preparing winter flower-bud material for microscopic examination, unbroken series of sections cannot be obtained by the ordinary methods of dehydrating, imbedding, etc. This is due to the diverse but characteristic structures found in a resting bud. These structures include heavy impervious protective scales, dense mats of hairs between the young leaves, flowers and bracts, and a delicate embryonic tissue at the tip. A continuous series of sections may be obtained by (1) making use of extremely sharp tungsten needles to remove the more minute scales and bracts, especially those between the embryonic flowers; (2) soaking the material for at least two months in 70% alcohol; (3) using *n*-butyl alcohol instead of absolute alcohol and xylol; (4) keeping the material continuously at low pressures; and (5) using an alcoholic stain. Each of these additional steps helps, but all are necessary for completely satisfactory results. A method by which the special tungsten needles may be made is described. The continuous treatment at low pressures is made possible by using a two ounce bottle fitted with a capillary tube and stopcock. The stains which proved most satisfactory were alcoholic solutions of safranin and acid fuchsin.

The proper preparation of certain winter buds for serial sectioning is a problem that presents many difficulties especially if the buds are flower buds. Papers dealing with the histology of the winter buds always mention the fact that imbedding, etc. could not be carried out by the ordinary methods. As considerable work has been done in the Botanical Laboratories at Dalhousie University with buds in their resting condition, and some of the difficulties in technique overcome, it was thought that it would be of assistance to others to record the methods that proved successful.

A brief description of the structural features which render the usual methods ineffective with winter buds should help to make clear why certain procedures are necessary. These special features can be grouped under three headings, (i) the heavy impervious scales that surround the bud; (ii) the dense mat of hairs between the young leaves, flowers and bracts, and (iii) the delicate condition of all the embryonic tissue at the tip. The heavy scales on the outside are usually covered with a wax-like coating and impregnated with a protective substance which is so efficient that they are impervious not only to water but also to any of the liquids ordinarily used in histological technique. In addition, the tissue of these scales is so tough and the cells so thick walled, that they cut with difficulty and the sections will not adhere

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to the slide. The hairs around the inner leaves, flowers and bracts do not become completely wetted even with such a liquid as ether. As a result, numerous small bubbles of air are retained between these hairs, even after prolonged and frequent use of the air pump. This air may make imbedding and sectioning impossible. The embryonic tissue of the young flowers or of the growing tip is often so delicate that it is macerated by the most dilute solutions of such softening reagents as hydrofluoric acid. Thus, when one wishes to study these delicate tissues, it is not practical to soften the outer scales and hairs by the usual methods. Owing to the combination and close association of these three structural features, modification of the ordinary routine technique and addition to it are necessary before one can obtain properly stained sections in an unbroken series.

Although the ordinary methods described in the textbooks when taken by themselves are quite inadequate, it must be clearly understood that all these ordinary methods are also necessary. For instance, the buds must be opened, the outer scales removed, all collections must be subjected to prolonged treatment under the air pump after both killing and washing. The special precautions outlined in this paper are necessary in addition to all the ordinary methods. The usual technique, however, is so well known and is so well described in the textbooks, that it is not necessary to review it here.

The winter buds for which the methods were worked out were chiefly from the apple, but buds from various species of the Ericaceae were also used. The killing fluid used was 1% chromacetic. Non-aqueous killing fluids were unsatisfactory, because of their high vapor pressure.

The additional precautions that were found necessary involved the following: dissection with special needles; prolonged soaking in a weak alcohol; the use of *n*-butyl alcohol throughout; a continuous exposure to low pressures; and an alcoholic stain. The subject will be discussed under each of these headings.

Dissection with Special Needles

If the bud is a flower bud of a species such as the apple, the original dissection must include the removal of the small bracts between the embryonic flowers. It is impossible to do this with even the sharpest steel needles. Their points are comparatively so blunt that injury to the delicate tissues and flower primordia is bound to result. Such material as finely drawn out glass, etc. is too brittle to remove these rather tough scales. Dr. G. H. Henderson of the Physics Department at Dalhousie provided us with extremely sharp tungsten needles. With these it was possible to remove the smallest structure without injury to the parts to be examined. Dr. Henderson has very kindly described the method of making these needles, as follows.

"These needles are best made from tungsten wire of about $\frac{1}{2}$ mm. diameter. Cut off about 2 cm. of the wire by careful grinding on an emery wheel. More forcible cutting, as by pliers, usually results in splitting the end of the cut-off wire, rendering it unfit for use. Mount the cut-off piece of tungsten wire

in a metal handle. A convenient way is to insert the wire into a short piece of brass tubing or drilled rod and squeeze or pound the brass so as to grip the wire firmly.

"The needle is sharpened by holding the end of the tungsten wire in a bath of molten sodium nitrite. This is easily done by melting the sodium nitrite carefully in a crucible, by a Bunsen burner. The crucible may be supported on a tripod and the needle by a clamp on a retort stand so that about 2 mm. of the wire dips into the molten nitrite. Small bubbles will be seen to come from the tungsten. After from 5 to 15 minutes the needle will be eaten away to a sharp point. It is saving of time if several needles are so treated at the same time. Caution should be exercised in heating the crucible just sufficiently so that bubbles are given off freely; greater heating may result in ignition.

"The progressive sharpening of the point may be followed from time to time by lifting the needle from the bath and examining it under a magnifying glass. Experience will soon enable one to obtain the degree of sharpness desired. When this is reached the needle should be washed under the tap and allowed to dry; it is then ready for use. It is hardly necessary to add that the finer the point the more carefully must it be used. No finer point should be chosen than is necessary for the work in hand."

Prolonged Soaking in a Weak Alcohol

It was found that after the buds had stood for a considerable time (at least two months) in various liquids, they became infiltrated with paraffin much more easily, and cut in a more satisfactory manner. When the processes of dehydrating and imbedding were started and completed in the ordinary way immediately after washing, it was impossible to obtain good serial sections. Even running the material through the dehydrating series very slowly did not help much. This failure to cut and ribbon properly was due to the large number of air bubbles still retained in the mat of fine hairs, and to the tough resistant character of the woody tissue at the base of the bud. The prolonged soaking helped with both these difficulties, for during immersion in a suitable liquid the air gradually disappeared from between the hairs, (presumably it was slowly dissolved by the liquid) and at the same time the tissues of the woody base became sufficiently softened to make cutting possible. Various liquids were used, but the most satisfactory proved to be alcohol of from 50 to 75%. Distilled water or a weaker alcohol resulted in too extensive maceration. The minimum time required for both the removal of the air and the softening of the tissues was two months. A longer period is better, and winter buds kept in 70% alcohol for two years were not injured, and cut perfectly.

Use of n-Butyl Alcohol

Removal of the air by dissolving it is a slow process. If these minute bubbles of gas can be dislodged and floated away by a liquid, much time is saved. This bodily displacement of the small air bubbles is apparently a

surface tension problem. Various liquids with a low surface tension were used. Most of them proved more or less unsatisfactory. The one that did prove satisfactory was *n*-butyl alcohol. The series used was as follows:—

Number of solution	Water, %	Ethyl alcohol, %	<i>n</i> -Butyl alcohol, %
1	85	15	0
2	70	30	0
3	50	40	10
4	30	50	20
5	15	50	35
6	5	45	50
7	0	25	70
8	0	0	100

If the tissue was washed in water, it was started in Solution No. 1 and left about 24 hours in each solution. After two washes of pure *n*-butyl alcohol, the material was placed in the oven and paraffin chips added to the *n*-butyl alcohol. From then on, the tissue was carried through the usual number of steps and periods of time to pure paraffin. For preserving the tissue in the *n*-butyl alcohol mixture, Solution No. 4 should be used.

Treatment at Low Pressures

The usual treatments at low pressure during and after killing, washing, dehydrating and imbedding were found to be inadequate. Also there are obvious difficulties associated with the prolonged use of the air pump over a volatile liquid such as an alcohol. The ordinary paraffin baths offered on the market for imbedding "*in vacuo*" were unsatisfactory for various reasons, the chief being that they took care of the imbedding period only. Also the use of any apparatus of large volume in which the air pressure must be reduced to a low value necessitates the continuous operation of an air pump. This is impractical as the volatile dehydrating fluids would evaporate to dryness. In order to subject the tissue continuously to a low pressure, a special but very simple piece of apparatus was devised as follows. An ordinary two ounce specimen bottle is fitted with a rubber cork through which is inserted a glass stopcock. This stopcock must have both inlet and outlet of capillary tubing. If the tubing has a larger bore, it is very difficult to prevent leakage.

Immediately the tissue was washed, it was placed in one of these bottles fitted with the rubber cork and stopcock. Both cork and stopcock were well sealed with a good grade of stopcock grease. The pressure of the air in the bottle was then reduced until the liquid commenced to boil. The stopcock in the tube was then turned off and the air pump disconnected. The tissue was left in this apparatus under low pressure until it was transferred to the next solution. Tests were made with a mercury manometer and it was found that the pressure inside the two ounce bottle had not changed perceptibly even after the apparatus had stood for one or two days. Each time

the liquid was changed the air pump was connected to the bottle and the pressure was reduced as described. In the treatments with the various members of the *n*-butyl alcohol series, the tissue was always kept under low pressure in each solution for at least 24 hours. When the stage for paraffin chips was reached, the whole apparatus was put in the oven. At the pure paraffin stage the same apparatus was used, placed in the oven, and the material kept under a low pressure just as at the other stages. If the bottle was allowed to cool while the air was being exhausted, the paraffin assumed an opaque creamy appearance. This apparently did no harm, and the paraffin became perfectly transparent when melted again in the oven. But better results were obtained if the paraffin was not allowed to solidify. This was accomplished by standing the bottle containing the liquid paraffin in hot water during evacuation. When this was done, the paraffin did not become opaque and creamy and a greater quantity of gas was drawn off. When the tissue was in small pieces, dehydrating fluids and paraffin were saved by standing the bottle and stopcock diagonally during evacuation.

Obviously the pressure inside the bottle could not be reduced below the vapor pressure of the liquid in which the tissue was immersed, and as it was necessary for the tissue to be continuously immersed, there was always a minimum below which the pressure could not be reduced. The procedure outlined above, however, kept the tissue subjected to this minimum pressure from the time it was washed until it was imbedded, the only exceptions being the brief periods during which the liquids were changed. If the material had been standing for some months at atmospheric pressure in either Solution No. 4 of the *n*-butyl alcohol series or in 70% ethyl alcohol, it was found best to run the tissue back to 15% ethyl alcohol and start the low pressure treatment as outlined above from the beginning of the *n*-butyl alcohol series.

Alcoholic Stains

Even after taking all these precautions the sections did not adhere well to the glass if the slides were placed in water. Hence it was found advisable to use alcoholic stains. The most satisfactory were safranin in 50% alcohol for nuclei, and acid fuchsin in 70% alcohol for a general stain.

Each of the five treatments outlined above was tested by itself, and resulted in a decided improvement in the results obtained, but to obtain completely satisfactory results, all five had to be followed.

Details of procedure would differ with different tissues and also with the varying customs of individual investigators, but it might be of assistance to give in outline the method we usually used. It is as follows:

Dissection of buds with special needles.

Dissected buds placed in 1% chrom-acetic killing fluid and immediately subjected to low pressure under the air pump for about 30 min.

Killing fluid changed and buds left in fresh killing fluid for 24 hr.

Washed in running water 24 hr.

Air pump with tissue in water. About 30 min.

For each stage from now on (except when otherwise stated) the tissue must be kept under low pressure in the 2 oz. bottle fitted with capillary tube and stopcock.

15% ethyl alcohol. 24 hr.

30% ethyl alcohol. 24 hr.

50% ethyl alcohol. 24 hr.

70% ethyl alcohol. 24 hr.

After 24 hr. under low pressure, the buds are left in 70% ethyl alcohol and stored in a corked bottle at atmospheric pressure for at least two months. Ethyl alcohol was used up to this point and for storing the tissue, because it is cheaper than *n*-butyl alcohol. Preparatory to imbedding in paraffin, it was found necessary for satisfactory results to return the stored material to water or a weak alcohol, at low pressure, for 24 hr., before running it through the graded alcohols, 24 hr. each, at low pressure, in the following series:—

From storage in 70% ethyl alcohol.

50% ethyl alcohol. 24 hr.

30% ethyl alcohol. 24 hr.

Solution No. 1 *n*-butyl alcohol series. 24 hr.

Solution No. 2 *n*-butyl alcohol series. 24 hr.

Solution No. 3 *n*-butyl alcohol series. 24 hr.

Solution No. 4 *n*-butyl alcohol series. 24 hr.

Solution No. 5 *n*-butyl alcohol series. 24 hr.

Solution No. 6 *n*-butyl alcohol series. 24 hr.

Solution No. 7 *n*-butyl alcohol series. 24 hr.

Solution No. 8 *n*-butyl alcohol series. 24 hr. (1st immersion).

Solution No. 8 *n*-butyl alcohol series. 24 hr. (2nd immersion).

Paraffin chips are then added and the whole apparatus placed in the oven and left there for about 3 hr.

Pure paraffin, 1st immersion. Not less than 4 hr.

Pure paraffin, 2nd immersion. Long enough to make a total of 24 hr. in pure paraffin. It is necessary to change the paraffin once to remove the surplus *n*-butyl alcohol.

Imbed buds in paraffin. It is of course necessary to imbed in the open at atmospheric pressure.

Tissue cut in ribbons, mounted on slides and stained in an alcoholic stain.

Acknowledgments

The apple buds used in this work were collected by the staff at the Laboratory of Plant Pathology, Kentville, N.S. The National Research Council of Canada provided a trained technician for three months to help with the routine part of the work. With the help of this assistant it was possible to experiment in technique methods, and arrive at the results outlined above.

RESISTANCE OF WINTER WHEATS TO HESSIAN FLY¹

BY W. R. FOSTER² AND C. E. JEFFERY³

Abstract

Stage of growth at the time of the spring emergence of the Hessian fly appears to account for the differential resistance of varieties of winter wheat at Saanichton, British Columbia. Varieties more advanced in growth appear to be more resistant or freer from infestation than varieties less advanced. There was a positive correlation ($r = +0.84$) between the number of days to maturity and infestation, and a negative correlation ($r = -0.63$) between the height of the wheat and infestation on April 1, about the time the fly emerges.

The varieties of winter wheat grown on the Dominion Experimental Station at Saanichton, B.C. showed culm infestation as follows: (i) Practically free from infestation (0-4%) Dawson's Golden Chaff O. 24, Dawson's Golden Chaff (O.A.C. 61), Kanred \times Dawson's Golden Chaff, Kharkov \times Dawson's Golden Chaff, Imperial Amber, O.A.C. 104, Red Rock and Triplett; (ii) Moderately infested (30-80%) Crail Fife, Forty-fold, Hybrid 128, Hussar, Oro, Yanward, and Yaroslav; (iii) Heavily infested (85-100%) Albit, Golden Sun, Jenkins \times Redit, Kharkov, Marshal Foch, Martin, Redit, Sun, Victor, White Odessa and Yeoman.

Nitrate of soda, superphosphate, and a complete fertilizer broadcast or drilled had no significant effect on Hessian fly damage.

Introduction

In 1934 at Saanichton, British Columbia, Hessian fly infested some winter wheat varieties up to 100% in five replicated and distributed plots, while others were left practically free. Owing to such a heavy infestation and also because a number of the varieties of winter wheat have not been previously tested for resistance, it was thought advisable to record the percentage of infestation, number of days to maturity, and yield. An association was indicated between infestation and number of days to maturity, so height and type of growth were noted on April 1, about the time the fly emerges. In 1935 practically no infestation took place in any variety. The results of 1934 were partially substantiated when a light infestation took place in 1936.

Abundant evidence of a differential infestation of wheat varieties has been reported by McColloch and Salmon (2), Rockwood and Reeher (4), Cartwright and Weebe (1), Painter, Salmon and Parker (3), and others.

According to McColloch and Salmon (2) the Hessian fly may and often does, lay as many, or more, eggs on a variety of winter wheat that remains practically free from infestation as on one that becomes heavily infested.

Our observations agree with Rockwood and Reeher (4) in that the Hessian fly infestation of winter wheat in the Pacific Northwest has been limited almost without exception to that occurring in the spring.

Hessian fly investigation is not one of our projects and we do not propose to carry the work further, but it seems worthwhile to publish the results obtained to date.

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Methods

The winter wheat variety plots used in this experiment consisted of three rod rows replicated five and four times in 1934 and 1936 respectively. The yields were taken on the centre rows, while the percentage of infestation was obtained by examining each culm in the first 10 feet of the right side row.

The dates of seeding the winter wheat plots were October 18, 1932, October 12, 1933, September 26, 1934, and September 26, 1935.

The fertilizer plots consist of five rod rows replicated four times and with check replicated eight times. The yield was taken on the centre row.

Correlations were obtained by using the Pearson's product-deviation method. The formula used was:

$$r = \frac{\sum xy}{n \sigma_x \sigma_y}$$

For measuring the probable variation in r the formula was:

$$P E_r = 0.6745 \frac{1 - r^2}{\sqrt{n}}$$

For obtaining the probable errors given in Table I the formula used for each variety was:

$$0.6745 \times \sqrt{\frac{\sum x^2}{n(n-1)}}$$

Experiments and Results

The percentage of culm infestation caused by the Hessian fly and the number of days to maturity of different varieties of winter wheat grown on the Experimental Station at Saanichton in 1934 are shown in Table I. Culms of the varieties Sun and Yeoman were infested 100% in the five replicated plots, while Egyptian Amber was not infested at all. The relation between the percentage of culms infested and number of days to maturity is shown by the high positive correlation ($r = +0.845 \pm 0.05$).

TABLE I

PERCENTAGE OF CULMS INFESTED AND NUMBER OF DAYS TO MATURITY OF DIFFERENT VARIETIES OF WINTER WHEAT AT SAANICHTON IN 1934

Variety	Culms infested, %	Days to maturity
Albit	97.1 \pm 0.9	287
Berkeley Rock	11.9 \pm 1.4	271
Crail Fife	66.7 \pm 3.0	270
Dawson's Golden Chaff O. 24	0.2 \pm 0.1	267
Dawson's Golden Chaff O.A.C. 61	0.2 \pm 0.1	270
Egyptian Amber	0.0 \pm 0.0	271
Fortyfold	46.6 \pm 3.1	270
Golden Sun*	97.2 \pm 0.8	289
Hussar	53.1 \pm 1.0	277
Hybrid 128	78.8 \pm 4.3	285
Imperial Amber	0.2 \pm 0.1	267
Jenkins \times Ridit	88.9 \pm 2.1	284
Kanred \times Dawson's Golden Chaff	2.4 \pm 0.6	275
Kharkov \times Dawson's Golden Chaff	3.8 \pm 0.7	273
Kharkov	97.7 \pm 0.5	281

TABLE I—*Concluded*PERCENTAGE OF CULMS INFESTED AND NUMBER OF DAYS TO MATURITY OF DIFFERENT VARIETIES OF WINTER WHEAT AT SAANICHTON IN 1934—*Concluded*

Variety	Culms infested, %	Days to Maturity
Marshal Foch	98.9 \pm 0.6	290
Martin	98.7 \pm 0.5	284
O.A.C. 104	0.4 \pm 0.1	270
Oro	47.1 \pm 3.0	275
Red Rock	0.4 \pm 0.1	267
Ridit	90.5 \pm 2.4	271
Sun	100.0 \pm 0.0	290
Triplet	0.4 \pm 0.3	272
Victor	99.4 \pm 0.3	289
V.I.S. 131*	86.1 \pm 3.6	287
White Odessa	90.0 \pm 2.6	282
Yanward	32.2 \pm 5.2	277
Yaroslav	69.5 \pm 2.9	279
Yeoman	100.0 \pm 0.0	290

* Hybrid of Dawson's Golden Chaff \times Sun.

Table II shows the height of varieties of winter wheat on April 1, about the time the flies emerge from the stubble and lay eggs on winter wheat (4), and the percentage of culm infestation by the Hessian fly in 1934 and 1936. Varieties practically free from infestation in 1934 were also free in 1936. The relation between the height of winter wheat varieties on April 1 (about

TABLE II

THE HEIGHT OF WINTER WHEAT VARIETIES ON APRIL 1, AND PERCENTAGE OF CULM INFESTATION BY THE HESSIAN FLY IN 1934 AND 1936 AT SAANICHTON

Variety	Height April 1, in.	1934 Culms infested, %	1936 Culms infested, %
Albit	15.0	97.0	15.7
Baldmin	11.5	97.3	12.8
Berkeley Rock	20.5	11.9	4.7
Crail Fife	16.0	66.7	16.0
Dawson's Golden Chaff O. 24	22.0	0.2	0.0
Dawson's Golden Chaff O.A.C. 61	22.0	0.2	0.2
Egyptian Amber	24.0	0.0	0.0
Fortyfold	15.0	46.6	0.0
Golden Sun	14.0	97.2	11.1
Hussar	14.0	53.1	0.7
Hybrid 128	16.0	78.8	12.5
Imperial Amber	20.5	0.2	0.0
Jenkins \times Ridit	10.0	88.9	11.8
Marshal Foch	10.0	98.9	15.5
Martin	23.5	98.7	14.5
O.A.C. 104	15.5	0.4	0.0
Oro	14.0	47.1	13.4
Ridit	15.0	90.5	15.1
Red Rock	23.0	0.4	0.4
Sun	10.5	100.0	15.0
Triplet	13.0	0.4	2.2
Victor	10.0	99.4	11.6
White Odessa	16.0	90.0	12.0
Yaroslav	16.5	69.5	3.2
Yeoman	9.0	100.0	12.7

the time the fly emerges) and the percentage of culm infestation is shown by the negative correlation ($r = -0.628 \pm 0.083$). Both correlations indicate that varieties of winter wheat that have reached a certain stage of growth at the time of the spring emergence escape serious injury.

The yield in bushels per acre of winter wheat varieties from 1933 to 1936 inclusive and the average yield for these four years are shown in Table III. All of the varieties of wheat were practically free from infestation in 1933 and 1935, while in 1934 infestation was very heavy and in 1936 it was light. Table IV shows the average yield of varieties of winter wheat tested from

TABLE III

THE YIELD, IN BUSHELS PER ACRE, OF WINTER WHEAT VARIETIES FROM 1933 TO 1936, INCLUSIVE, AT SAANICHTON

Variety	1933	1934	1935	1936	Average
Albit	24.0	7.4	34.0	31.2	24.0
Baldmin	23.8	19.2	60.1	35.4	34.6
Berkeley Rock	26.9	12.0	49.8	32.0	35.2
Crail Fife	34.3	9.9	46.7	32.3	30.8
Dawson's Golden Chaff O. 24	39.3	16.8	51.5	36.5	36.0
Dawson's Golden Chaff O.A.C. 61	38.1	14.6	63.2	42.3	39.5
Egyptian Amber	32.5	10.5	48.5	32.8	31.1
Fortyfold	37.9	11.2	52.0	41.1	35.5
Golden Sun	41.3	10.4	49.4	27.4	32.1
Hussar	23.2	18.0	47.8	43.2	33.0
Hybrid 128	31.0	11.0	37.5	20.1	24.9
Imperial Amber	25.2	20.3	55.1	42.1	35.7
Jenkins X Redit	24.8	12.6	28.2	26.6	23.0
Marshal Foch	33.9	9.5	43.5	26.7	28.4
Martin	26.2	15.7	27.0	26.6	23.9
O.A.C. 104	29.4	25.3	49.4	35.2	34.8
Oro	33.2	19.5	28.3	24.0	26.2
Redit	26.8	15.8	23.4	19.1	21.3
Red Rock	35.9	28.6	48.8	42.1	38.8
Sun	36.4	6.9	40.3	20.5	26.0
Triplet	32.9	27.1	43.3	34.8	35.0
Victor	33.4	10.8	47.6	29.4	30.3
White Odessa	33.5	17.4	38.5	25.5	29.2
Yaroslav	33.1	16.5	41.2	36.1	31.7
Yeoman	30.8	0.0	32.7	26.9	22.6

TABLE IV

THE AVERAGE YIELD OF WINTER WHEAT VARIETIES IN SAANICHTON

Variety	No. of years tested	Average yield, bu. per acre
Dawson's Golden Chaff O. 24	11	42.9
Golden Sun	11	37.8
Imperial Amber	9	40.8
Marshal Foch	11	35.6
O.A.C. 104	11	38.8
Red Rock	11	40.4
Sun	11	33.0
Victor	11	35.2
Yeoman	11	29.6

9 to 11 years. The varieties that are resistant to Hessian fly infestation, Dawson's Golden Chaff, Imperial Amber, O.A.C. 104, and Red Rock, are also the four leading varieties in yield. Their high yield is probably partly due to their resistance to Hessian fly.

The effect of different fertilizers, broadcast and drilled, on the yield of Sun Wheat heavily infested by the Hessian fly in 1934 at Saanichton is shown in Table V. Nitrate of soda, superphosphate and a complete fertilizer did not appear to have any significant effect on Hessian fly damage.

TABLE V

THE EFFECT OF DIFFERENT FERTILIZERS ON THE YIELD OF SUN WHEAT HEAVILY INFESTED BY HESSIAN FLY IN 1934, AT SAANICHTON

Treatment		Yield, bu. per acre	
Nitrate of soda, 125 lb. per acre, broadcast		7.73	
Nitrate of soda, 125 lb. per acre, drilled		5.63	
Superphosphate, 250 lb. per acre, broadcast		6.73	
Superphosphate, 250 lb. per acre drilled		6.68	
Complete	{ Nitrate of soda 125 lb. per acre Superphosphate 250 lb. per acre Muriate of potash 50 lb. per acre	Broadcast	
Complete	{ Nitrate of soda 125 lb. per acre Superphosphate 250 lb. per acre Muriate of potash 50 lb. per acre		Drilled
Check, no fertilizer		7.83	

Discussion of Results

The high positive correlation ($r = +0.84$) between number of days to maturity and infestation, and the negative correlation ($r = -0.63$) between height of plants on April 1 (about the time the Hessian fly begins to emerge and lay its eggs on winter wheat) and infestation, indicate that stage of growth may account for the differential resistance of winter wheat varieties at Saanichton. The 1934 data are particularly attractive owing to the marked difference in culm infestation which ranged from 0-100% among varieties grown in five replicated and distributed plots. Furthermore the 1936 data although only ranging from 0 to about 15% tend to substantiate the 1934 results. Rockwood and Reeher (4) state that, "Fall-sown wheat, if seeded early enough to get a good start before cold weather sets in, usually makes enough growth before the spring emergence of the Hessian fly to escape serious injury". Our results tend to support this statement, and show further that the probable cause for the differential resistance of varieties is the stage of growth at the time of the spring emergence of the Hessian fly. Those varieties most advanced in growth by the time of the spring emergence of the fly appear to be more resistant than those less advanced. Both Rockwood and Reeher's and our results were obtained in the Pacific Northwest. In Kansas, where conditions are quite different, Painter, Salmon and Parker (3) state that, "With the exception of the two characters, grain texture and colour of stem,

fly resistance does not seem to be associated with any other commonly observed character of the wheat plant, such as time of maturity, awn type, glume or kernel colour".

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PHYSIOLOGIC FORMS OF LOOSE SMUT OF WHEAT¹

By W. F. HANNA²

Abstract

Four physiologic forms of loose smut of wheat have been found in Manitoba. Two of these forms were collected in the field, one on Reward and the other on Mindum. The two other forms appeared in the course of artificial inoculations in the greenhouse. The origin of physiologic forms of loose smut of wheat is discussed. It is considered that one of the forms that appeared in the course of the greenhouse inoculations may have resulted from a mutation. Evidence is put forward which indicates that different physiologic forms occur in Eastern and Western Canada. None of the 13 varieties of wheat used in the inoculation experiments proved to be resistant to all physiologic forms. The inoculation of Reward, Marquis, Garnet, and Pentad \times Marquis with their own spores for four generations did not result in appreciably increasing the infections on these varieties. It was also shown that the healthy Reward plants that are sometimes present in a population grown from artificially inoculated seed are not resistant to loose smut, but have escaped infection because of faulty inoculation.

Introduction

In 1931 a brief report (6) was made of the occurrence in Manitoba of two physiologic forms of loose smut of wheat, *Ustilago Tritici* (Pers.) Rostr. One of these forms was collected on the durum wheat Mindum, and the other on the common wheat Reward. Prior to the publication of this report, Rodenhiser (10, 11) had found that there were distinct differences in the cultural characters of collections of *U. Tritici* originating in different localities. In all, 14 of these cultural forms were described, but no attempt was made to correlate cultural behavior with differences in pathogenicity. Since the publication of Rodenhiser's work extensive experiments with monosporidial cultures of the smut fungi have been made by a number of workers. In view of the results of these investigations cultures of loose smut of wheat that differ in appearance would not necessarily be expected to exhibit differences in pathogenicity.

The first announcement of the occurrence of physiologic forms of loose smut of wheat appears to have been made by Piekenbrock (8). By inoculating a number of varieties of wheat with several collections of spores, he was able to identify two physiologic forms. In wheat crosses made by him, resistance to loose smut was found to be inherited recessively. Piekenbrock's work was continued by Grevel (4) who studied 19 collections of loose smut from Germany and 29 from foreign countries. This material yielded four physiologic forms, three of which were present in the German collections, while the fourth form originated from a collection of loose smut sent from Turkey.

The results of inoculation experiments published by Tapke (14) in 1929 suggested the probable existence of physiologic forms of loose smut of wheat in the United States. A further contribution to the subject was made by

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Ruttle (13) who noted a striking difference between the pathogenicity of spores collected on Reward wheat in Manitoba and those collected on Honor wheat in the state of New York. Recently, Radulescu (9) made a study of physiologic forms of loose smut in Rumania. The winter wheats in that country were apparently attacked by but one physiologic form, but collections from the summer wheats were found to belong to three distinct forms. These forms corresponded with Forms 2, 3 and 4, previously identified by Grevel.

Experimental

OUTLINE OF INVESTIGATION

The experiments described in the following pages were begun in 1929. They were undertaken to determine (i) the pathogenicity of collections of loose smut from certain varieties of wheat grown in Manitoba; and (ii) the extent to which the pathogenicity of a loose-smut collection can be modified by propagating it for several generations on a particular variety of wheat. If, in each original smut collection, there were present only a single physiologic form homozygous for pathogenicity, any subsequent changes in behavior might be regarded as the result of mutation. If, on the other hand, each original collection consisted of a mixture of two or more physiologic forms, the selective effect of a number of wheat varieties might be expected eventually to separate each collection into its component forms. This selective effect would probably manifest itself by a progressive increase in virulence on certain varieties and a corresponding decrease on others.

METHODS

With the exception of certain field inoculations, which will be referred to later, the floral inoculations and the growing of the inoculated seed to determine the degree of loose-smut infection were carried out in the greenhouse. This work was commenced in the fall of 1929 and was completed in February, 1936. The first inoculations were made with spores collected in the field in 1929 on the varieties Mindum, Kota, and Reward. All subsequent inoculations were made with spores that were the direct descendants of these three collections.

The method of designating the different lots of inoculum, and the relationships among them are shown in Fig. 1. The three original collections made on Mindum, Kota, and Reward were designated respectively Mi, K, and R. The descendants of these three collections were assigned letters and numbers to indicate the varieties through which they had passed. The following abbreviations of varietal names were employed: Mindum (Mi), Kota (K), Reward (R), Marquis (M), Garnet (G), Pentad \times Marquis ($P \times M$), Renfrew (Ren), Pentad (P), and Khapli (Kh). Spores labelled (RG₁), for example, were collected from Garnet which had been inoculated with spores from Reward. Similarly, those marked (R M₁ P₁) were collected from Pentad inoculated with spores from Marquis which, in turn, had been inoculated with spores originally collected on Reward.

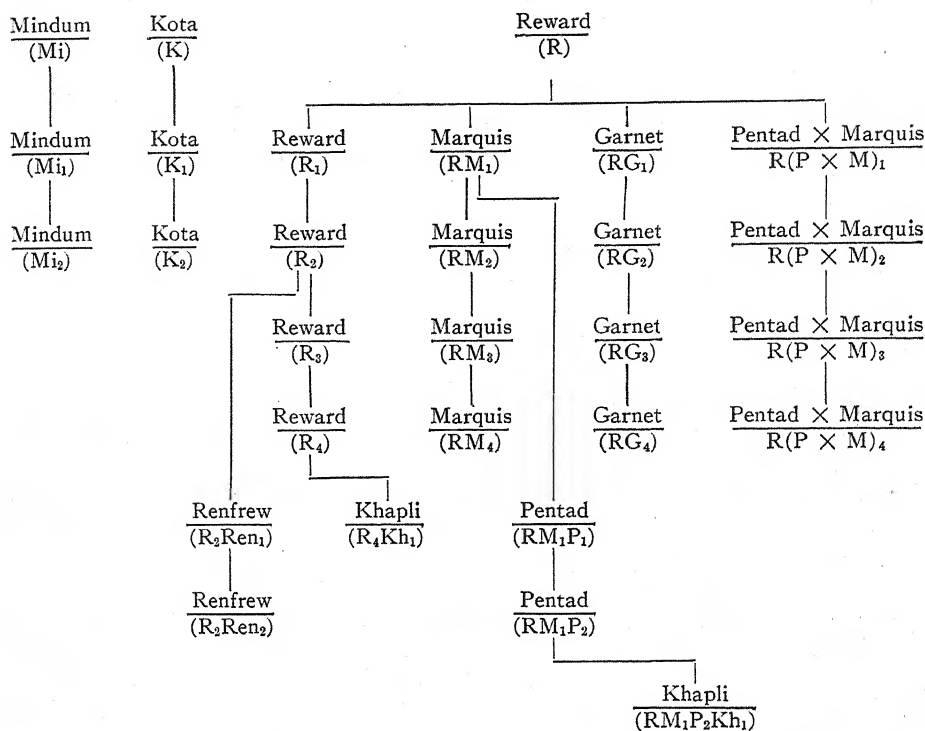


FIG. 1. Pedigree of loose smut of wheat collections used in inoculation experiments.

Varieties to be inoculated were grown in pots of soil in the greenhouse. As soon as extrusion of the anthers had commenced the heads were prepared for inoculation by removing with a pair of forceps the small central florets and those at the top and bottom of the spike. The tips of the forceps were then dipped in a vial of spores and the glumes of each floret were forced slightly apart so as to allow spores to fall on the tip of the stigma. The inoculated heads were not bagged, but during the time of seed-setting both temperature and humidity in the greenhouse were kept relatively high.

The percentage infection in the inoculated seed was determined by sowing about one hundred seeds in pots or flats of soil in the greenhouse. When the plants had headed out, the total number of plants and the number of smutted plants from each lot of seed were recorded. The conditions under which different lots of plants were grown were kept as uniform as possible, but variations due to seasonal changes in light intensity could not be prevented.

The literature dealing with the effect of growing conditions on the appearance of smutted heads in wheat grown from infected seed was reviewed recently by the writer (5). The evidence on this subject was somewhat contradictory, but it seemed to indicate that the degree of loose-smut infection is not appreciably influenced by the conditions under which plants are grown. The critical period appears to be at the time of flowering, and Tapke (15) has

shown that if a high humidity is maintained at that time susceptible varieties, when inoculated with viable spores, become heavily infected. A comparison of the results in some of the following tables will show that varying percentages of loose smut were obtained when the same variety was inoculated at different times with the same collection of spores. This variation probably resulted from slight differences in the technique of inoculation, and from lack of uniformity in environmental conditions at the time of inoculation and throughout the period of plant growth. The relative importance which should be attached to each of these factors is at present unknown.

PATHOGENICITY OF THREE COLLECTIONS OF LOOSE SMUT

Eleven varieties of wheat were inoculated with loose-smut spores of the three collections, R, K, and Mi, originally made in 1929 from the varieties Reward, Kota, and Mindum. Subsequently the same varieties were re-inoculated with the first, second, and third generations of the R spores, and with the first and second generations of the K and Mi spores. With the exception of the red *durum* variety, Pentad, and the amber *durum*, Mindum, all of the varieties belong to the *vulgare* group of wheats. The percentages of infected plants resulting from these inoculations are given in Table I.

TABLE I

INOCULATION OF WHEAT VARIETIES WITH SUCCESSIVE GENERATIONS OF LOOSE-SMUT SPORES
(Greenhouse inoculations; Winnipeg)

Variety	Per cent infection									
	R	R ₁	R ₂	R ₃	K	K ₁	K ₂	Mi	Mi ₁	Mi ₂
Reward	77	59	86	97	71	87	92	25	1	0
Garnet	91	15	25	37	63	63	88	8	0	0
Marquis	37	30	71	96	25	74	85	3	0	0
Renfrew	0	0	4	2	0	0	0	0	0	0
Marquillo	47	52	86	50	74	96	95	—	0	0
Ceres	72	76	81	89	69	77	100	4	0	0
Preston	4	0	3	12	1	0	0	0	0	0
L. Club	62	2	72	50	53	92	74	0	0	0
Kota	45	32	50	37	65	86	97	—	0	0
Mindum	0	0	0	0	0	0	0	72	77	80
Pentad	0	0	0	0	1	0	0	42	18	2

The results of these inoculations indicate very clearly the existence of two physiologic forms of loose smut. One of these forms, present in the collections from Reward and Kota, infected most of the common wheats, but was unable to infect Mindum, and produced only a slight infection on Pentad in one inoculation. The other form, collected on Mindum, infected this variety heavily, and in the first inoculation produced 42% infection on Pentad, but gave only a light infection on a few of the common wheats. In inoculations with the two subsequent generations of spores, Mi₁ and Mi₂, the high infection on Mindum was maintained, whereas the infection on Pentad diminished, and that on the four common wheats, Reward, Garnet, Marquis, and Ceres, was

reduced to zero. This apparent change in pathogenicity was probably due to progressive purification of the Mi inoculum by repeated passage through the same host. A similar phenomenon has already been noted by Dillon-Weston (2, 3) in his experiments on bunt of wheat.

The only varieties possessing a high degree of resistance to both physiologic forms of loose smut were Renfrew and Preston. These varieties, however, gave low percentages of infection when inoculated with certain collections of spores. The significance of these light infections will be discussed later.

PURIFICATION OF LOOSE-SMUT COLLECTIONS

Other experiments were made to determine the extent to which a collection of loose-smut spores can be separated into several physiologic forms by repeated passage through particular varieties of wheat. The following varieties of wheat were used in these experiments: Reward, Garnet, Marquis, Pentad \times Marquis, Pentad, Mindum, and Khapli. The variety designated as Pentad \times Marquis is a stem-rust-resistant hybrid which was produced at the Dominion Rust Research Laboratory. The first inoculations were made with the R strain of spores which had been collected in the field on Reward. This inoculated seed was sown in the greenhouse, and spores were gathered from the smutted plants of Reward, Marquis, Garnet, and Pentad \times Marquis to be used for the second inoculation of the seven varieties. These spore collections were labelled respectively R_1 , RM_1 , RG_1 , and $R(P \times M)_1$. Subsequently, all seven varieties were inoculated with the second, third, and fourth generations of spores produced on the four varieties of wheat. The results of all the inoculations are given in Table II.

Inoculation of the varieties listed in Table II with successive generations of their own spores did not result in a progressive increase in infection. Different generations of the same strain of spores, when used to inoculate the variety from which they were collected, sometimes gave widely different percentages of infection. For example, infection on Reward with R_1 spores was only 59%, whereas with R_3 spores it was 97%. This increase in infection was probably not due to the greater virulence of the R_3 spores, although it is possible that this may have contributed in some measure to the increase. Since the inoculations were made at different times and under somewhat different conditions it is probable that the variations in infection should be attributed to these factors rather than to changes in the pathogenicity of the spores.

If the virulence of a strain of spores with respect to a given variety could be enhanced by repeated passage of the spores through that variety it might be expected that the highest mean infection on the variety would be secured by inoculations with several generations of its own spores. By referring to the mean infections resulting from inoculations with the four strains of spores, as given in Table II, it will be seen that the highest infections on Reward and Garnet were obtained by inoculating with spores produced respectively on Reward and Garnet. However, the infection on Reward with Reward spores

was only slightly heavier than with Garnet spores. On the other hand, the highest infections on Marquis and Pentad \times Marquis were secured with spores from Garnet, and not with spores from these two varieties. The relatively high mean infections produced on all four varieties of common wheat by Garnet spores suggests that this strain of inoculum may have been more virulent than the others. Apart from this possible exception, the results in Table II do not lend support to the view that the pathogenicity of loose-smut inoculum can be progressively enhanced by repeated passage through the same host.

OCCURRENCE OF NEW PHYSIOLOGIC FORMS

In the discussion of Table I, attention was directed to the light infections that appeared when Renfrew and Preston were inoculated with certain collections of spores. Other light infections occur again in Table II, on Pentad and Khapli. Spores were collected from infected plants of some of these varieties and were used to re-inoculate the varieties from which they had come. In this way it was hoped to isolate and purify any new physiologic forms of loose smut that might have appeared. Renfrew was inoculated with spores gathered from the Renfrew plants on which R_2 spores had produced 4% of smut (Table I). When this inoculated seed was grown, 87% of the plants produced from it were smutted. Spores (R_2Ren_2) from these plants were then used to inoculate 13 varieties of wheat. The results of these inoculations are given in Table III. From the infections obtained on Renfrew, Ceres, and Preston it is apparent that the R_2Ren_2 spores belong to a new physiologic form, quite different from the two forms represented by the R_2 and Mi_2 spores.

Spores from infected plants of Khapli produced by inoculation with R_4 spores (Table II) gave negative results when Khapli was re-inoculated with them. Khapli was also re-inoculated with spores from plants of this variety that had become infected when inoculated with RM_1P_2 spores (Table III). This inoculation gave an infection of only 7% on Khapli, whereas the RM_1P_2 spores had given an infection of 14%.

When Pentad was re-inoculated with spores gathered from the one per cent of infected plants resulting from inoculation with RM_1 spores (Table II), an infection of 24% was obtained. Spores from these plants, designated as RM_1P_2 , were then used to inoculate the 13 varieties of wheat listed in Table III.

TABLE III
REACTION OF VARIETIES OF WHEAT TO PHYSIOLOGIC
FORMS OF LOOSE SMUT
(Greenhouse inoculations; Winnipeg)

Variety	Per cent infection			
	R_2	Mi_2	R_2Ren_2	RM_1P_2
Reward	86	0	83	0
Garnet	25	0	80	0
Marquis	71	0	81	0
Renfrew	4	0	66	0
Marquillo	86	0	24	0
Ceres	81	0	0	0
Preston	3	0	88	0
Little Club	72	0	0	0
Kota	50	0	0	0
Pentad \times Marquis	25	0	43	0
Pentad	0	2	0	57
Mindum	0	80	0	36
Khapli	0	64	0	14

By referring to this table it will be seen that the infection on Pentad was increased to 57%. Apparently, therefore, the RM_1P_2 spores are distinctly different in pathogenicity from the RM_1 spores from which they originated. They differ also from the Mi_2 spores in the extent to which they infect Pentad, Mindum, and Khapli. In view of these differences the RM_1P_2 strain of inoculum must be regarded as a distinct physiologic form of loose smut.

SELECTION OF REWARD FOR RESISTANCE TO LOOSE SMUT

In the inoculation experiments already referred to it will be noted that among even the most susceptible varieties a certain number of plants failed to become infected. The occurrence of these smut-free plants need occasion no surprise when it is considered that ordinary methods of inoculation cannot be relied upon to give perfect infection. It is possible, nevertheless, that a few of the plants escaped infection, not because of faulty inoculations, but because of their inherent resistance. Standard varieties of wheat are not necessarily pure lines with respect to loose-smut reaction and it is possible that within each variety there are one or more strains differing considerably in smut reaction. If this hypothesis were correct, repeated selection from smut-free plants through several generations might be expected to produce pure lines having greater resistance to loose smut than would be found in a mixed population of the same variety.

With this end in view a number of smut-free plants of Reward, which had appeared in a population grown from artificially inoculated seed, were re-inoculated with spores of the Reward strain of loose smut. The seed from these plants was bulked and, when grown in 1930, it was found that of the plants produced from 141 seeds, 86 or 61% were smutted. Thirty-seven of the 55 plants that had remained smut-free were then chosen for re-inoculation, and one head of each plant was inoculated with Reward spores (R_1 spores) collected from the smutted plants. The inoculated seed from each head was then harvested and sown separately to determine the percentage of infection.

The seed from 16 of the 37 heads produced only smutted plants, so no further selection from these lines was possible. By referring to the first four columns of Table IV it will be seen that seed from the remaining 21 heads produced varying percentages of smutted plants. One head of each of the smut-free plants was then inoculated with spores (R_2) collected from the infected plants, and the seed from each of these heads was grown separately. This procedure was repeated with most of the lines up to the F_3 generation. At this point nearly all of the lines showed from 90 to 100% infection, and it was decided to confine further inoculations to the progeny of Head 16, which had given rather low infection in the F_1 and F_2 generations. This line has been continued to the F_3 generation, which shows 67%.

The data in Table IV give some idea of the extent of variation in infection that may occur when a variety is inoculated at different times and under somewhat different conditions with the same strain of loose smut. As might be expected, the variation in infection was greatest in the first and second

TABLE IV
SELECTION OF REWARD WHEAT IN FIVE GENERATIONS FOR RESISTANCE TO LOOSE SMUT

Head No.	F ₁ Generation			F ₂ Generation			F ₃ Generation			F ₄ Generation			F ₅ Generation		
	Total plants	Smutted plants	Smut, %	Total plants	Smutted plants	Smut, %	Total plants	Smutted plants	Smut, %	Total plants	Smutted plants	Smut, %	Total plants	Smutted plants	Smut, %
6	11	10	91	8	5	63									
11	1	0	0	16	16	100									
13	3	1	33	14	14	100									
15	17	15	88	9	6	67	18	18	100						
16	19	10	53	78	33	42	259	7	100						
17	13	12	92	26	6	86	7	73	92	59	49	83	39	26	67
18	9	4	44	34	17	50	102	91	89						
20	19	13	68	7	2	29	30	30	100						
21	9	7	78	21	10	48	75	71	95						
22	12	6	50	20	16	80	18	18	100						
23	12	6	50	33	37	62	151	139	92						
25	12	4	33	5	4	80									
28	2	1	50	5	4	80									
29	17	14	82	19	13	68	42	42	100						
31	14	0	0	84	47	56	168	158	94						
32	11	6	55	39	24	62	97	92	95						
33	13	4	31	65	52	80	69	64	93						
34	14	10	71	28	13	46	87	86	99						
35	11	9	82	20	15	75	21	19	91						
36	2	1	50	13	11	85	9	9	100						
37	10	9	90	11	7	64	29	26	90	59	49	83	39	26	67
	*340	*251	74	584	359	62	1261	1181	94						

* Included in this total are the progeny of 16 heads, all of which (109 plants) were smutted.

generations when most of the lines were composed of only a small number of plants. In the F_3 generation, when greater numbers of plants were dealt with, the infection percentages tend to become more uniform. By the end of the third generation the entire progeny of 24 of the original 37 plants had been destroyed by loose smut, and the progeny of 10 others showed infections of 90% or more. Apparently, therefore, in plants such as wheat, which are normally self-pollinated, the presence among infected plants of a small number of healthy individuals signifies accidental escape from infection rather than inherent resistance.

OCCURRENCE OF DIFFERENT PHYSIOLOGIC FORMS IN EASTERN AND WESTERN CANADA

The four physiologic forms of loose smut of wheat referred to in Table III all originated in the province of Manitoba. No survey has yet been made of the physiologic forms occurring in other parts of Canada, but there is sufficient indirect evidence to indicate that certain forms occur more frequently in some districts than in others.

In the years 1933, 1934, and 1935 several standard varieties of wheat and a number of stem-rust-resistant hybrids that were being tested for yield and quality in Western Canada were artificially inoculated with loose smut. These wheats were grown in the field at Winnipeg and, during the flowering period, heads were inoculated with spores of the Reward strain of loose smut. The inoculations were made in the same way as the greenhouse inoculations already referred to. The seed harvested from these plants was later sown in pots of soil in the greenhouse to determine the degree of infection.

In 1933, Dr. L. H. Newman, the Dominion Cerealist, arranged to have several of the same varieties of wheat grown at the Dominion Experimental Station, Charlottetown, P.E.I., where they were artificially inoculated with loose-smut spores which had been gathered at that station. Some of this

TABLE V
REACTIONS OF VARIETIES OF WHEAT INOCULATED WITH LOOSE SMUT AT CHARLOTTETOWN,
P.E.I., AND WINNIPEG, MAN.
(Field inoculations)

Variety	Per cent infection			
	Charlottetown spores	Winnipeg spores		
	1933	1933	1934	1935
Marquis	12	66	58	78
Reward	42	91	90	79
Ceres	0	71	56	86
Huron		0	1	0
Garnet			68	72
Early Triumph			3	0
(D.C. × H-44) × (D.C.) A.303-1	0	85		
Pentad × Marquis 12-10-3	55	0		

inoculated seed was then sent to the Dominion Rust Research Laboratory, Winnipeg, where it was sown in the field in the spring of 1934. The percentage infection in this seed and in seed of some of the same varieties inoculated at Winnipeg with Reward spores in the years 1933, 1934, and 1935 is given in Table V.

The variety Huron, when inoculated at Winnipeg, proved to be immune in 1933 and 1935, and was only lightly infected in 1934. Unfortunately, this variety was not inoculated at Charlottetown, but a published report (7) indicates that it is susceptible to loose smut in Prince Edward Island. The varieties Ceres, (D.C. \times H-44) \times (D.C.) A.303-1, and Pentad \times Marquis 12-10-3 behaved quite differently towards the two kinds of spores, and their reactions alone show conclusively that the spores used in the inoculations at Winnipeg and Charlottetown represent different physiologic forms.

Discussion

The experiments described in the preceding pages confirm the findings of other investigators that there occur in nature numerous physiologic forms of loose smut of wheat. Different forms, however, tend to predominate in different districts, depending upon the varieties of wheat that are grown. In Prince Edward Island, where Huron wheat is grown, a form attacking this variety has appeared, whereas in Western Canada there are forms attacking Reward, Marquis, Garnet, and Mindum, the varieties most commonly cultivated in the prairie provinces. This tendency of the parasite towards physiologic specialization suggests that new varieties of wheat, unless they possess a high degree of resistance to a number of physiologic forms, may be expected, eventually, to become affected by loose smut.

Although the fact of the occurrence of physiologic forms of loose smut of wheat has been clearly established, comparatively little is known of the manner and frequency of their appearance. Given a mixture of physiologic forms, the host plant may act as a screen in separating them into different parasitic strains. This, however, is a purely mechanical separation and has no relation to the origin of new physiologic forms. In the light of present-day knowledge new forms might be expected to arise by mutation, or by the genetic recombination of two existing forms.

In a recent paper Roemer and Kamlah (12) discussed the manner in which mixtures of physiologic forms of loose smut are reduced to pure lines by repeated passage through the same host. They consider the possibility of the host plant exerting a certain modifying action upon the parasite, resulting in a temporary change in its pathogenicity. According to this hypothesis continued association of host and parasite would result in a more congenial relationship between the two, but would not alter the genetic constitution of the parasite. If the virulence of the parasite could be enhanced in this way, breeding for resistance to loose smut would be ineffectual. It has been well established, however, that resistance to loose smut is inherited in accordance with Mendelian laws. Roemer and Kamlah conclude, therefore, that the

increase in virulence which is sometimes associated with the continued culturing of a collection of loose smut on one host is merely the result of purification of the inoculum by selection.

In the section of the present paper dealing with the data summarized in Table II it was pointed out that the inoculation of Reward, Garnet, Marquis and Pentad \times Marquis with their own spores for four generations did not lead to increases in the percentages of smut on these varieties. Failure to increase the infection on Reward, Garnet, and Marquis might be attributed to the fact that these varieties were so susceptible to the parent collection of smut (R) that heavier infections could scarcely be expected. Such an explanation, however, fails to account for the behavior of Pentad \times Marquis, which proved to be moderately resistant throughout all of the inoculations. If in the parent collection of inoculum there had been present a few spores of a physiologic form to which Pentad \times Marquis was highly susceptible, four generations of selections on this variety would probably have purified and increased it. In the absence of evidence to this effect it may be concluded that the parent collection of spores did not contain such a strain.

It might be expected that continuous cultivation on the same farm of a particular variety of wheat, such as Reward, would result in a few years in the isolation by natural selection of one or more physiologic forms of loose smut highly specialized on that variety. In contrast with the uredospores of the rust fungi which are carried long distances by the wind, with the result that every year new physiologic forms may be introduced into a district, the spores of loose smut of wheat have an effective spread of probably less than a mile. Moreover, uredospores may initiate infection on any part of the plant on which they fall, whereas the spores of loose smut of wheat can only cause floral infection. Consequently, as the distance from the source of inoculum increases the density of loose-smut spores diminishes and the frequency of infection falls off rapidly. It is for this reason that the rogueing of smutted heads from a field as soon as they appear has been recommended as a method of controlling loose smut of wheat. The peculiar manner in which loose smut of wheat causes infection and is carried over from one season to another in the same stock of seed may account for the failure of the inoculation experiments referred to in Table II to effect a separation of the parent collection of spores into different physiologic forms. These spores, which were gathered in a single field of Reward, had probably already been reduced by natural selection to a pure line.

The two physiologic forms, R_2Ren_2 and RM_1P_2 (Table III), appeared during the course of the inoculation experiments. The form designated as R_2Ren_2 attacks Reward heavily and could without difficulty maintain itself on this variety. Consequently, it might have originated as an impurity in the parent collection of R spores. The RM_1P_2 form, however, does not attack Reward and for that reason could scarcely have been present among the R spores originally collected on that variety. The manner of its appearance and its host range suggest that it arose as a mutation from the parent R strain.

Investigations on the inheritance of morphological and physiological characters in *U. Tritici* have been limited because of the difficulty of securing haploid cultures of this fungus. The technique that has been employed with such success in the study of inheritance in the sporidium-producing smuts cannot be adapted to species such as *U. Tritici*, the spores of which on germination produce hyphae and not sporidia. Recently, however, Christensen (1) announced that he had secured haploid cultures of *U. Tritici*. Floral inoculations with a single haploid culture gave negative results, but when the inoculations were made with certain pairs of such cultures a high percentage of kernels became infected. This work has opened up a new and interesting field of research. It will now be possible to obtain lines of *U. Tritici* that are homozygous for pathogenicity, and the relative contributions to the origin of new physiologic forms of this fungus made by hybridization and mutation may be accurately determined.

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THE BEHAVIOR OF PAIRED MONOSPOROUS MYCELIA OF *FOMES ROSEUS* (ALB. & SCHW.) COOKE AND *FOMES SUBROSEUS* (WEIR) OVERH.¹

BY IRENE MOUNCE² AND RUTH MACRAE²

Abstract

Fomes roseus and *F. subroseus* are heterothallic and bipolar. With one exception, complete interfertility exists between haploid mycelia derived from different sources. The exception is of particular interest since it shows that two cultures of *F. roseus* from widely separated sources possess one interfertility factor in common. *F. roseus* and *F. subroseus* may be differentiated on the basis of their spore characters. The failure to obtain clamp connections in any of the many pairings of a haploid mycelium of *F. roseus* with a haploid mycelium of *F. subroseus* only serves to emphasize that these two fungi are distinct.

Introduction

Fomes roseus (Alb. and Schw.) Cooke and *F. subroseus* (Weir) Overh. are two fungi which, in young growing specimens, have their context and pore surface pinkish or rose-colored and which may, at times, resemble one another quite closely (Plate I). For many years *F. subroseus* was either confused with *F. roseus* or erroneously named *Trametes carnea* Nees. In 1923 Weir (6) pointed out the distinguishing characters of each fungus and made the new species *Trametes subrosea* to include those usually thinner forms with "darker-colored context, and the conspicuous narrow zonate and radiate fibrillose surface of the pileus" and with narrowly elongated to allantoid spores. In 1933 Overholts (3) transferred this species to the genus *Fomes* and in 1935 (4) again stressed the difference in spore characters: the spores of *F. roseus* (Figs. 1-6) are "elongate-ellipsoid, hyaline, $5-7 \times 2.5-3.5\mu$ " while those of *F. subroseus* (Figs. 7-12) are "narrow-cylindric, hyaline, slightly curved, $4-7 \times 1-2\mu$ ".

Snell, Hutchinson, and Newton (5) in their study of temperature relations of *F. roseus* and *F. subroseus* (*Trametes subrosea*) obtained such a definite difference in temperature response that they could readily distinguish the two species in culture. These results were in agreement with Weir's conclusions.

Because of the confusion which has existed it seemed worth while to study the behavior of monosporous mycelia of both *Fomes roseus* and *F. subroseus* in order to apply the clamp-connection criterion for the identity of species which Vandendries (7) has stated as follows: "Si les haplontes de deux carpophores sauvages sont toujours et indéfiniment fertiles entre eux ces deux carpophores appartiennent à une même espèce." A preliminary note on this work appeared in the Report of the Dominion Botanist for 1930.

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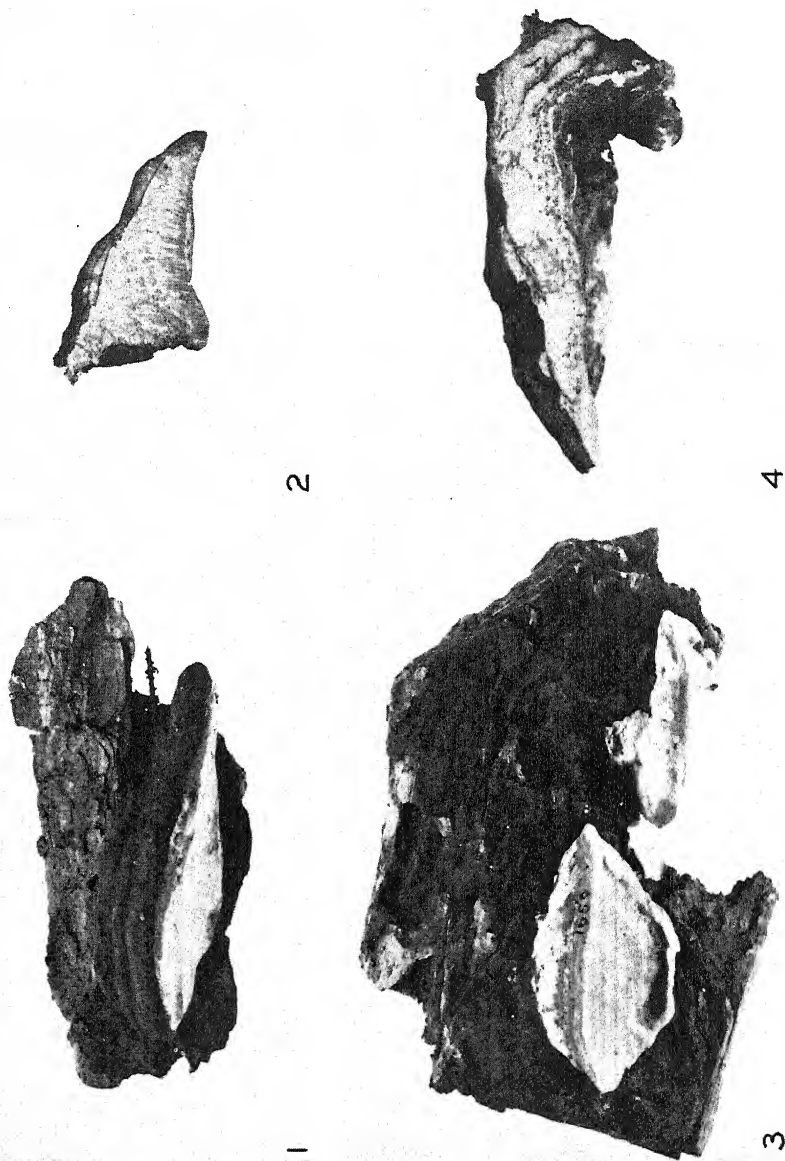


FIG. 1. *Fomes roseus*. Sporophore No. 821. FIG. 2. *F. roseus*. Vertical section of Sporophore No. 2355. FIG. 3. *F. subroseus*. Sporophore No. 1658. FIG. 4. *F. subroseus*. Vertical section of Sporophore No. 1639. Figures 1-2 are two-thirds natural size, Figures 3-4 are natural size.

Isolation of Single Spores

Under the name of *Fomes roseus*, *Trametes subrosea*, or *T. carnea* the cultures listed in Table I were available in the collection at Ottawa.

TABLE I

Culture No.	Host	Locality
368		(From Dr. E. E. Hubert)
376		(From Dr. E. E. Hubert)
693	<i>Abies balsamea</i>	Fredericton, N.B.
1000	?	(From Centraalbureau voor Schimmelcultures, Baarn)
1036	<i>Pseudotsuga taxifolia</i>	Vancouver, B.C.
1046	<i>Pseudotsuga taxifolia</i>	Vancouver Island, B.C.
1299	<i>Picea rubra</i>	Cranberry Lake, N.Y.
1300	Wood of coniferous tree	Oswego County, N.Y.
1404	<i>Picea canadensis</i>	Timagami, Ont.
1449	<i>Picea canadensis</i>	Timagami, Ont.
1639	? <i>Pseudotsuga taxifolia</i>	Benton County, Ore.
2355	<i>Picea canadensis</i>	Gaspé County, P.Q.
2378	<i>Pseudotsuga taxifolia</i>	Point Atkinson, B.C.
2391	Wood of coniferous tree	Keewatin, Ont.
2392	Wood of coniferous tree	Keewatin, Ont.

Sporophores were obtained from cultures grown on prune or malt agar or on small blocks of Douglas fir (*Pseudotsuga taxifolia*) which had been surface sterilized in acetic acid fumes (1) then placed on the slanted surface of prune agar in 250 cc. flasks. Basidiospores were collected on a sterile cover-slip placed beneath a fruit-body, a drop of sterile distilled water was added, and the whole smeared over the surface of lactose gelatine in Petri plates. After germination, isolations were made by cutting out, with a fine needle under the compound microscope, a square of gelatine containing a single spore and placing it in a tube of malt agar. Single spore isolations were made from each of the 15 cultures listed in Table I.

Paired Monosporous Mycelia

From the Same Source

Clamp-connections did not develop on any monosporous mycelium so pairings were made. The results of a series of all possible pairings of 15 haploid mycelia of culture No. 2391 are shown in Table II, and of 15 haploid mycelia of culture No. 2392 in Table III. The plus sign indicates the presence of clamp-connections and the minus sign their absence. Similar series of pairings were made using haploid mycelia from each of the 15 cultures listed above. In every case the haploid mycelia could be divided into two groups. Clamp-connections were formed in every pairing of a member of one group with a member of the other group. The fungi from which the cultures were made are, therefore, heterothallic and bipolar.

TABLE II

THE RESULTS OF PAIRING IN ALL POSSIBLE
COMBINATIONS 15 MONOSPOROUS MYCELIA
OF *Fomes roseus* No. 2391

A															a									
1 2 3 5 11 12 14															4 6 7 8 9 10 13 15									
A	1	-	-	-	-	-	-	+	+	+	+	+	+	+	+									
	2	-	-	-	-	-	-	+	+	+	+	+	+	+	+									
	3	-	-	-	-	-	-	+	+	+	+	+	+	+	+									
	5	-	-	-	-	-	-	+	+	+	+	+	+	+	+									
	11	-	-	-	-	-	-	+	+	+	+	+	+	+	+									
	12	-	-	-	-	-	-	+	+	+	+	+	+	+	+									
	14	-	-	-	-	-	-	+	+	+	+	+	+	+	+									
a	4	+	+	+	+	+	+	-	-	-	-	-	-	-	-									
	6	+	+	+	+	+	+	-	-	-	-	-	-	-	-									
	7	+	+	+	+	+	+	-	-	-	-	-	-	-	-									
	8	+	+	+	+	+	+	-	-	-	-	-	-	-	-									
	9	+	+	+	+	+	+	-	-	-	-	-	-	-	-									
	10	+	+	+	+	+	+	-	-	-	-	-	-	-	-									
	13	+	+	+	+	+	+	-	-	-	-	-	-	-	-									
	15	+	+	+	+	+	+	-	-	-	-	-	-	-	-									

TABLE III

THE RESULTS OF PAIRING IN ALL POSSIBLE
COMBINATIONS 15 MONOSPOROUS MYCELIA
OF *Fomes subroseus* No. 2392

A									a						
1 3 4 6 8 9 13 14 2 5 7 10 11 12 15															
A	1	-	-	-	-	-	-	-	+	+	+	+	+	+	+
	3	-	-	-	-	-	-	-	+	+	+	+	+	+	+
	4	-	-	-	-	-	-	-	+	+	+	+	+	+	+
	6	-	-	-	-	-	-	-	+	+	+	+	+	+	+
	8	-	-	-	-	-	-	-	+	+	+	+	+	+	+
	9	-	-	-	-	-	-	-	+	+	+	+	+	+	+
a	13	-	-	-	-	-	-	-	+	+	+	+	+	+	+
	14	-	-	-	-	-	-	-	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	-	-	-	-	-	-	-
	5	+	+	+	+	+	+	+	-	-	-	-	-	-	-
	7	+	+	+	+	+	+	+	-	-	-	-	-	-	-
	10	+	+	+	+	+	+	+	-	-	-	-	-	-	-
	11	+	+	+	+	+	+	+	-	-	-	-	-	-	-
	12	+	+	+	+	+	+	+	-	-	-	-	-	-	-
	15	+	+	+	+	+	+	+	-	-	-	-	-	-	-

From Different Sources

Pairings of haploid mycelia from one source with haploid mycelia from each of the other sources show that the 15 cultures can be arranged into two groups as follows:

Group A

1404
1449
2355
2391
1299

Group B

368 1639
376 2378
693 2392
1000 1036
1300 1046

Haploid mycelia from any culture in Group A are completely interfertile with haploid mycelia from every other culture in that group; that is, they belong to one and the same species. There is one interesting exception to this statement in the behavior of haploid mycelia of Culture No. 2355 when paired with haploid mycelia of Culture No. 2391. It does not, however, alter in any way this general conclusion and will be dealt with separately at the end of the paper. Similarly haploid mycelia from any culture in Group B are completely interfertile with haploid mycelia from every other culture in that group, that is, they, too, belong to one and the same species. Three hundred and eighty-one pairings were made among members of Group A and four hundred and seventy among Group B and Tables IV and V are typical of the results obtained. But though 502 pairings have been made, no

haploid mycelium of Group A has been found that would form clamp-connections when paired with a haploid mycelium of Group B, and Tables VI-VII are typical.

TABLE IV

THE RESULTS OF PAIRING FIVE MONOSPOROUS MYCELIA OF *F. roseus* No. 2391 WITH TWO MONOSPOROUS MYCELIA OF *F. roseus* No. 1449

		2391				
		1	2	3	4	5
1449	3	+	+	+	+	+
	7	+	+	+	+	+

TABLE V

THE RESULTS OF PAIRING FIVE MONOSPOROUS MYCELIA OF *F. subroseus* No. 2392 WITH TWO MONOSPOROUS MYCELIA OF *F. subroseus* No. 693

		2392				
		1	2	3	4	5
693	1	+	+	+	+	+
	3	+	+	+	+	+

TABLE VI

THE RESULTS OF PAIRING FIVE MONOSPOROUS MYCELIA OF *F. roseus* No. 2391 WITH TWO MONOSPOROUS MYCELIA OF *F. subroseus* No. 693

		2391				
		1	2	3	4	5
693	1	-	-	-	-	-
	3	-	-	-	-	-

TABLE VII

THE RESULTS OF PAIRING FIVE MONOSPOROUS MYCELIA OF *F. subroseus* No. 2392 WITH TWO MONOSPOROUS MYCELIA OF *F. roseus* No. 2391

		2392				
		1	2	3	4	5
2391	1	-	-	-	-	-
	4	-	-	-	-	-

The results of all of these pairings are shown graphically in Table VIII in which the plus sign indicates that clamp-connections were formed in every pairing of a monosporous mycelium from the one source with a monosporous mycelium from the other source, and the minus sign that they were absent. Here the members of Group A are labelled *Fomes roseus* and the members of Group B, *Fomes subroseus*.

That the cultures in Group A are *Fomes roseus* and those in Group B are *F. subroseus* may be shown by reference to the shape of the spores obtained from the various cultures that were used. No spores from Cultures 1299, 1036, and 1046 are available at present, but for the rest, spores from Cultures 1404,

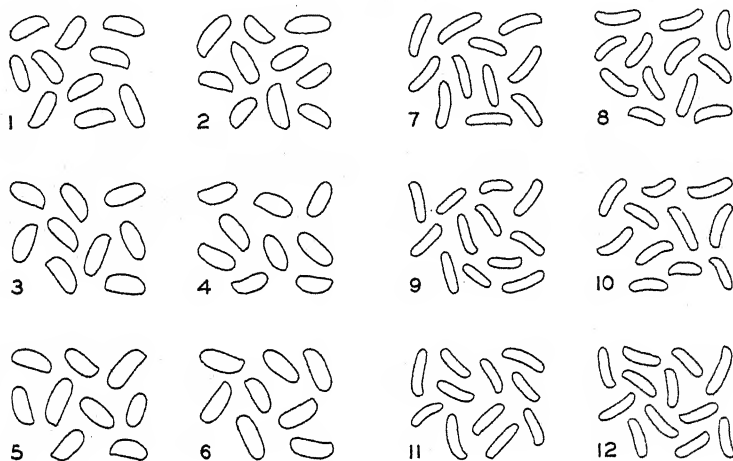
TABLE VIII

SUMMARY OF RESULTS OF ALL PAIRINGS SHOWING

- Complete interfertility of monosporous mycelia of *F. roseus*
- Complete interfertility of monosporous mycelia of *F. subroseus*
- Absence of clamp-connections in every pairing of a monosporous mycelium of *F. roseus* with a monosporous mycelium of *F. subroseus*

		<i>Fomes subroseus</i>										<i>Fomes roseus</i>				
		368	376	693	1000	1036	1046	1300	1639	2378	2392	1299	1404	1449	2355	2391
<i>Fomes subroseus</i>	368	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-
	376	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-
	693	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-
	1000	+	+	+	-	+	+	+	+	+	+	-	-	-	-	-
	1036	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-
	1046	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-
	1300	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-
	1639	+	+	+	+	+	+	+	-	+	+	-	-	-	-	-
<i>Fomes roseus</i>	2378	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-
	2392	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
	1299	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
	1404	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
	1449	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
	2355	-	-	-	-	-	-	-	-	-	-	-	+	+	+	±
	2391	-	-	-	-	-	-	-	-	-	-	-	+	+	+	±

1449, 2355, and 2391 are ellipsoid (Figs. 3-6) while those of Cultures 368, 376, 693, 1000, 1300, 1639, 2378 and 2392 are cylindric and slightly curved (Figs. 9-12). That basidiospores obtained from cultures are identical in color and shape with those of the original specimen has been shown already by the senior author (2). Further proof is given here since Figs. 1, 2, 7 and 8 were drawn from spores produced by wild fruit-bodies while Figs. 3, 4, 9



FIGS. 1-6. *Fomes roseus*. Spores elongate ellipsoid. FIGS. 1 and 3. Spores from a wild fruit-body No. 2391 and from a culture of that fruit-body respectively. FIGS. 2 and 4. Spores from a wild fruit-body No. 2355 and from a culture of that fruit-body respectively. FIGS. 5 and 6. Spores from cultures No. 1449 and No. 1404 respectively. FIGS. 7-12. *Fomes subroseus*. Spores narrow cylindric to slightly curved. FIGS. 7 and 9. Spores from a wild fruit-body No. 693 and from a culture of that fruit-body respectively. FIGS. 8 and 10. Spores from a wild fruit-body No. 2392 and from a culture of that fruit-body respectively. FIGS. 11 and 12. Spores from cultures 1000 and 1300 respectively. (Magnifications $\times 1025$.)

and 10 were drawn from spores produced in cultures made from these same fruit-bodies. The spores are indistinguishable. Hence the cultures in Group A which, as has been shown by pairing reactions, all belong to one species and all have ellipsoid spores, are cultures of *F. roseus* and those of Group B, which all belong to one species and have cylindric, slightly curved spores, are *F. subroseus*.

The complete interfertility of monosporous mycelia of *F. roseus* from different sources, the complete interfertility of monosporous mycelia of *F. subroseus* from different sources, and the failure to form clamp-connections in any of the 502 pairings of a monosporous mycelium from any of the five different *F. roseus* cultures with a monosporous mycelium from any of 15 different *F. subroseus* cultures is in accordance with the conclusion of Weir, Snell, Overholts, and others that these two fungi, *F. roseus* and *F. subroseus*, are distinct.

Pairings of *F. roseus* No. 2355 with *F. roseus* No. 2391

The unusual behavior of these two strains of *Fomes roseus*, from widely separated districts, may be explained by the hypothesis that they possess one factor in common. Culture No. 2355 was made from a sporophore collected in Gaspé Co., Quebec, on *Picea glauca* by Mr. A. W. McCallum, August 2, 1932. Culture No. 2391 was made from a sporophore collected at Keewatin, Ontario, on wood of a coniferous tree by Mr. M. Timonin, September 24, 1932. Sporophores were obtained in culture, monosporous mycelia were isolated, and pairings made. The results showed that, as usual, each fungus was heterothallic and bipolar, and that monosporous mycelia from these cultures of *F. roseus* were mutually fertile with monosporous mycelia of *F. roseus* from each of the other sources. It was somewhat surprising, therefore, to obtain results such as are shown in Table IX when monosporous mycelia of Culture No. 2355 were paired with monosporous mycelia of Culture No. 2391. To make sure that these results were not due to the age of the mono-

TABLE IX

THE RESULTS OF PAIRING IN ALL POSSIBLE COMBINATIONS
16 MONOSPOROUS MYCELIA OF *Fomes roseus* No. 2355
WITH 16 OF *Fomes roseus* No. 2391

		2391B															
		A								a							
		1	4	6	8	9	11	13	26	2	3	5	7	10	12	22	28
A ¹	1	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
	4	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
	8	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
	10	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
	12	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
	13	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
	16	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
	17	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
a ¹	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

sporous mycelia used, or to the conditions under which they had been kept or to other such factors, subcultures were made from the stock cultures of both No. 2355 and No. 2391. Fresh sporophores were obtained and new monosporous mycelia isolated from them. The results of pairing these mycelia are shown in Table IX. One factor, which we have designated A, was common to both fungi and hence no clamp-connections were formed in one whole group of pairings, where this factor was present in each of the monosporous mycelia being paired.

This unusual case was submitted to Dr. René Vandendries, Rixensart, Belgium, who has very generously given us permission to include his comment.

"Pour ce qui concerne le cas spécial que vous signalez, de *Fomes roseus*, une seule interprétation me paraît possible: il est certain, et votre tableau

l'indique, que les deux souches étrangères l'une à l'autre 2391 et 2355 ont le même facteur A. Pareil fait a été déjà signalé deux fois:

"Par *Hermann Brunswick* sur *Coprinus comatus*. Untersuchungen ueber die Geschlechts—und Kernverhältnisse bei der Hymenomyzeten—gattung *Coprinus*. (Botanische Abhandlungen 1924. Heft 5, page 109.)

"Par moi-même chez *Coprinus radians*. (Je mets moi-même en doute aujourd'hui, s'il s'agit de *C. radians* ou d'une espèce voisine. J'ai eu l'occasion de revoir des cultures de *C. radians* qui n'avaient pas l'aspect des premières et qui étaient homothalles.)

"Du mémoire 'Contribution nouvelle à l'étude de la sexualité des Basidiomycètes,' La Cellule, 3 juin 1924, j'extrait les conclusions générales suivantes:

"3. Les deux sexes qui apparaissent sur un carpophore, différent des deux sexes d'un carpophore étranger.

"4. Une mutation profonde a rendu un haplonte fertile pour tous ses congénères, stérile, au contraire, pour un certain nombre d'haplontes étrangers.

"5. Ce fait nouveau constitue une exception à la loi qui proclame la fécondité constante entre haplontes de carpophores étrangers.

"6. Le sexe de l'haplonte mutant a pu être identifié avec celui d'un groupe d'individus d'un autre carpophore.

"Vous vous trouvez devant un cas analogue. Ce qui le rend plus intéressant, c'est que l'existence du facteur A affecte tout un lot d'individus d'une sporée.

"Il diffère de ce que j'ai signalé par le fait que vos individus ne sont probablement pas stériles avec leurs congénères. La différenciation est donc moins prononcée que dans les deux premiers cas signalés.

"Quoi qu'il en soit, j'estime que l'existence de facteurs communs dans des souches étrangères doit être plus répandue que nous le supposons. Cette existence me semble une preuve admirable de la justesse des vues de Kniep, quand il a établi sa théorie des facteurs alléomorphes et de leur origine par mutations.

"Quelle que soit la multiplicité des souches, une origine commune doit fatalement se manifester par des cas d'indentité des facteurs sexuels. Si nous les trouvons rarement, c'est que la Nature est infinie et que nos moyens d'investigation devant cette immensité, est quantité négligeable. Il est miraculeux de pouvoir noter déjà trois cas pareils et c'est bien le vôtre qui est le plus intéressant."

Acknowledgments

The authors are much indebted to Mr. O. C. Anderson, Mr. I. L. Connors, Mr. E. J. Eliason, Dr. E. E. Hubert, Dr. H. S. Jackson, Mr. D. J. MacLeod, Mr. A. W. McCallum, Mr. M. Timonin, Dr. J. Westerdijk, and Dr. S. M. Zeller for their kindness in sending specimens and cultures, and to Dr. René Vandendries for his interest in our problem.

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VIRUS STUDIES

III. TOMATO DISEASES¹

BY W. NEWTON² AND H. I. EDWARDS³

Abstract

Single virus streak, potato virus X, streak virus X and aucuba mosaic (tobacco virus 6) were found causing diseases of tomatoes in commercial glasshouses in British Columbia during 1936. Single virus streak was the commonest disease although greater losses were caused by streak virus X. Aucuba mosaic was found in one case only, but was highly pathogenic. Potato virus X was present mixed with single virus streak, giving rare cases of mixed virus streak. Tomato mosaic (tobacco virus 1) was not present as a tomato disease.

Single virus streak serum did not give a precipitate when mixed with aucuba antigen, thus indicating that the viruses are distinct. However, a slight precipitate with tobacco virus 1 antigen did indicate distant relationship with this form. Although three strains of single virus streak could be distinguished by symptoms produced on tomatoes when inoculated simultaneously, these strains proved to be serologically identical.

Introduction

A survey of the glasshouses was made in 1936 to ascertain the nature and distribution of the virus diseases that affect tomato production in the coastal regions of British Columbia. Four distinct virus diseases were found and three of these, single virus streak, mixed virus streak and yellow or aucuba mosaic, have been reported as occurring in British Columbia (1). The fourth, streak X, has been described in the second paper of this series (5).

It may be important to distinguish between the diseases that naturally occur in tomatoes and those that can induce disease when transferred from other crops, *e.g.*, tomato mosaic (tobacco virus 1) has not been found affecting tomatoes, but it is quite common as a disease of tobacco in British Columbia. Again, a number of the local potato viruses are significantly pathogenic to tomatoes, but with the exception of potato virus X, no form that naturally occurs in local potatoes has been found in tomatoes.

Experimental

The commercial glasshouses of Vancouver Island and the lower Fraser Valley were inspected at intervals during the tomato production period. When symptoms that suggested virus infection appeared in the crop, leaf samples were taken. These were ground in a small amount of distilled water and transferred to healthy test plants by rubbing the leaf surfaces with a ground glass spatula moistened with the wet leaf pulp. Only four diseases could be sharply differentiated. These are briefly described in Table I.

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TABLE I

THE SYMPTOMS OF TOMATO DISEASES ON SPECIES OF *Solanaceae* AND THE LETHAL TEMPERATURES OF THE INFECTIVE PRINCIPLES

Disease	Lethal temp. 10 min.	Tomato	Tobacco (White Burley)	<i>N.</i> <i>gluti-</i> <i>nosa</i>	<i>Datura</i> <i>mete-</i> <i>loides</i>	<i>Datura</i> <i>stram-</i> <i>onium</i>	Petunia	Pepper
Single virus streak	80-85	m,n	L	L	L	L	m	L,m
Mixed virus streak	65-70 80-85	M,N	L,m	L,m	L,m	L,m	m	L,m
Streak X	65-70	M,N	Mr	M	m	m	m,n	m
Yellow mosaic	80-85	yM,N	L,yM	L	L	L	yM,n	yM

Explanation of symbols: m = mottle, ym = yellow mottle, L = local lesions, N = necrosis. Capitalization indicates that the symptom is very pronounced.

SINGLE VIRUS STREAK

The foliage symptoms of single virus streak in tomato ranged from a barely perceptible mottle to a pronounced mottle and crinkle, often accompanied by leaf distortion of the "fern leaf" type. The name of the disease suggests that necrosis of the foliage and stem are characteristic symptoms, but under conditions that approach the ideal for growth of tomatoes, streaked foliage is comparatively rare. Evidence was secured that several strains of single virus streak exist in British Columbia. Three forms were distinguished, characterized by leaf distortion (fern leaf), necrosis and mottle, and mottle only, respectively. However, the strains could only be distinguished by simultaneous inoculations. Under unfavorable growing conditions the least pathogenic form, namely, "mottle", could induce both necrosis and leaf distortion. As will be seen from Table II, the three forms are serologically indistinguishable.

Although single virus streak, tomato mosaic and yellow mosaic have similar lethal temperatures, 80-85° C., which suggests a relationship, nevertheless our serological study has indicated that the relationship is not close. Furthermore, on White Burley tobacco they are readily differentiated. The prominent local lesions induced by single virus streak are rarely followed by a systemic mottle except on very young tobacco seedlings. On the other hand, with tomato mosaic, a systemic mottle is the first symptom to appear. Although local lesions are a primary symptom of yellow mosaic, unlike single virus streak, the local lesions are invariably followed by a pronounced yellow mottle.

MIXED VIRUS STREAK

In agreement with Ainsworth *et al.* (1), mixed virus streak as it occurs in British Columbia was found to be caused by a mixture of single virus streak and potato virus X. Although the infected plants were found at widely different points, one from Vancouver Island and the other from the Lower

Fraser Valley, in both cases the components were identical. The potato virus X component could not be distinguished from the ordinary form isolated from healthy Up-to-date potatoes, on *N. glutinosa*.

Mixed virus streak can readily be distinguished from single virus streak. The development of local lesions on *N. glutinosa* within two days after leaf surface inoculations is a characteristic of both, but in mixed virus streak, the local lesions are followed by a mottle. The local lesions usually appear within two days and the mottle within a week.

STREAK X

In host range and in general properties streak X is similar to potato virus X, but on tomatoes it can readily be distinguished from the common strains of X. Streak X induces pronounced necrosis, while ordinary forms of potato virus X induce a faint mottle only. Although streak X is quite a common disease in commercial glasshouses, no case was discovered where streak X was the X component of mixed virus streak.

YELLOW OR AUCUBA MOSAIC

The culture of yellow or aucuba mosaic isolated in 1936 appeared to be more virulent or pathogenic on tomatoes than the form forwarded to and reported upon by Ainsworth, Berkeley and Caldwell (1), but otherwise no distinctive properties were found. The symptoms on tomatoes may be confused with the yellow form of tobacco virus 1. The mottles are very similar, but the yellow pigment is more prominent in yellow tobacco mosaic. Apart from the evidence in Table II that the two viruses are serologically distinct, yellow mosaic (tobacco virus 6) produces local lesions followed by a conspicuous yellow mottle on White Burley tobacco, but yellow tobacco virus produces a yellow mottle only.

SEROLOGICAL TESTS

Antiserum to single virus streak was prepared by inoculating rabbits with the expressed sap of tomato seedlings infected with a pure strain of this disease. The saps were first purified according to the method of Bawden and Pirie (2) before injecting them into rabbits, and before using them as antigen against the antisera so produced. The rabbit antiserum was further purified by centrifuging out the slight precipitate that forms when purified sap from healthy tomato seedlings is allowed to react with rabbit antiserum. The original and two additional strains of single virus streak were used as antigen, together with tomato mosaic (tobacco virus 1) and yellow or aucuba mosaic (tobacco virus 6). The results are summarized in Table II.

Normal rabbit serum in contact with the five virus antigens developed slight precipitates, but these precipitates were no more abundant than when antiserum was mixed with purified sap from healthy tomatoes. The possible error due to this precipitate was eliminated by pretreatment of the single virus

TABLE II
THE REACTION OF TOMATO VIRUSES AGAINST SINGLE VIRUS STREAK ANTISERUM

Antigen	Antigen dilution with saline	Antiserum dilution with saline	Antigen : antiserum precipitate
Single virus streak, Form 1	1 : 0	1 : 1	+
	1 : 10	1 : 1	+
	1 : 25	1 : 1	+
	1 : 0	1 : 10	?
	1 : 10	1 : 10	+
			+
Form 2	1 : 0	1 : 1	+
	1 : 10	1 : 1	+
	1 : 25	1 : 1	+
	1 : 0	1 : 10	+
	1 : 10	1 : 10	+
			+
Form 3	1 : 0	1 : 1	+
	1 : 10	1 : 1	+
	1 : 25	1 : 1	+
	1 : 0	1 : 10	+
	1 : 10	1 : 10	+
			+
Yellow mosaic (aucuba) tobacco virus 6	1 : 0	1 : 1	0
	1 : 10	1 : 1	0
	1 : 25	1 : 1	0
	1 : 0	1 : 10	0
	1 : 10	1 : 10	0
			0
Tomato mosaic (tobacco virus 1)	1 : 0	1 : 1	+
	1 : 10	1 : 1	?
	1 : 25	1 : 1	0
	1 : 0	1 : 10	+
		1 : 10	?
			?

streak antiserum with purified tomato sap from healthy tomatoes. The addition of an equal volume of purified sap to the rabbit serum was found to remove completely all non-specific antibodies.

The three strains of single virus streak reacted with a single form of antiserum, hence, in spite of their distinct symptoms on tomatoes they are apparently serologically identical. On the other hand, yellow, or aucuba mosaic failed to react with single virus streak antiserum and hence is serologically distinct. Slight evidence of a relationship between tomato mosaic and single virus streak was suggested by the faint precipitate that formed when tomato mosaic antigen was mixed with single virus streak antiserum.

Discussion

Single virus streak was by far the most common virus disease affecting tomatoes in the glasshouses of British Columbia. At least three strains appear to exist whose characteristic symptom expressions vary from a faint mottle to pronounced leaf distortion and necrosis. The symptoms found in

commercial glasshouses do not serve to identify particular strains owing to the profound alteration of symptoms by environment. A surprising feature of these investigations is that no case of tomato mosaic was found although few glasshouses were entirely free from virus diseases.

From the standpoint of economic importance, streak X is an important disease. Although it occurred less frequently than single virus streak, many cases were found where less than half the plants in a house yielded marketable fruit, owing to the presence of this disease. In spite of its prevalence and the fact that streak X belongs to the potato virus X group, it was never found associated with single virus streak or yellow mosaic in mixed virus streak. When streak X was combined experimentally with single virus streak or with yellow mosaic, both combinations resulted in highly pathogenic diseases. The synthetic disease formed by combining streak X and aucuba or yellow mosaic frequently induced death of tomato plants within ten days. This combination was more pathogenic than any form of "experimental streak" studied in this laboratory.

The form of mixed virus streak that naturally occurs in the commercial glasshouses of British Columbia is quite pathogenic but fortunately its occurrence is comparatively rare. Two cases only were discovered in 1936 although previously other cases were found. Owing to the general prevalence of single virus streak, it was not surprising that mixed virus streak was always a combination of single virus streak and the potato component rather than tomato mosaic and X.

Only one case of yellow or aucuba mosaic was discovered in the glasshouses of British Columbia during the 1936 survey. Although comparatively rare, it was more pathogenic to tomatoes than any other disease that naturally occurs in tomatoes.

The most surprising feature of these investigations was the discovery that single virus streak was serologically distinct from yellow or aucuba mosaic (tobacco virus 6) and tomato mosaic (tobacco virus 1). Most workers have assumed that the viruses that have lethal temperatures between 80 and 85° C. are closely related, partly because their host range is similar and again because no evidence has been presented until recently that they could be distinguished by serological means. Since the completion of these experiments, Chester (4) has shown that tobacco virus 1 and yellow or aucuba mosaic can be differentiated by serological methods. The ordinary technique does not distinguish between the two viruses but if the tobacco virus 1 serum is first absorbed with aucuba antigen and the precipitate is removed, this purified virus 1 serum will again react with virus 1 serum; or conversely, aucuba serum will again react with aucuba antigen after the absorption treatment with tobacco virus 1 antigen. On the other hand Chester (4) found that this residual precipitin reaction did not occur when the serum of masked strain of tobacco virus 1 was absorbed with the normal virus 1 antigen, or conversely, when the normal virus 1 antigen was absorbed with the masked

strain; hence masked tobacco virus may be defined as a true strain of ordinary tobacco virus, but aucuba or yellow mosaic may be considered as a fundamentally distinct virus. Our serological investigation indicates that the British Columbia form of yellow or aucuba mosaic is distinct from single virus streak and that single virus streak is only distantly related to tobacco virus, in spite of the fact that we did not use the absorption technique suggested by Beale (3) and applied by Chester. Unfortunately single virus streak antiserum only was used in our experiments and Chester did not study this virus.

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PRELIMINARY STUDIES OF THE TRANSFER OF FOUR STRAINS OF *DITYLENCHUS DIPSACI* (KÜHN 1858) FILIPJEV 1936¹

BY R. J. HASTINGS² AND WM. NEWTON³

Abstract

The bulb and stem nematode, *Ditylenchus dipsaci*, attacks narcissus, iris, red clover and strawberry in the Pacific Northwest. The isolations from each of these important crops are herein described as strains.

Preliminary studies of the transfer of these strains establish the existence of three strains of *D. dipsaci* in the Pacific Northwest, viz.:

(i) Red clover strain; characterized by causing swollen crowns and stunt in red clover seedlings.

(ii) Strawberry strain; characterized by a limited host range, swollen crowns in strawberry seedlings, and entrance into red clover seedlings without visible tissue reactions.

(iii) Narcissus and iris strain; characterized by a wide host range and entrance into clover and strawberry seedlings without visible tissue reactions.

No satisfactory technique of establishing the host range of the biological strains of *D. dipsaci* has been developed. The clamping of glass rings filled with a nematode suspension in moist pulverized peat to the foliage of test plants did not affect the test plants in a constant manner. The examination of seedlings after clarification in a lacto-phenol solution containing acid fuchsin gave more constant results. The seedlings were removed from infested soil shortly after they appeared above ground.

The reports of host specificity of the red clover strain were not confirmed, for the red clover strain entered white clover and alfalfa, hitherto considered resistant. Likewise, the reports of host specificity of the narcissus strain were not supported by our experimental results. The narcissus strain entered red clover and oats, also considered resistant hitherto.

Introduction

Ditylenchus dipsaci, the bulb nematode, is a serious pest on narcissus, iris, red clover and strawberry in the Pacific Northwest. Each of the isolations from these hosts is herein described as a biological strain. It is well known that *D. dipsaci* includes both varieties and biological strains. Steiner and Scott (10) have described the morphological differences of the four varieties, *dipsaci*, *amsinckiae*, *allocotus* and *communis*, but up to the present no morphological differences have been found in biological strains. A biological strain usually derives its name from a host upon which it has been confined for a number of generations, when some degree of specialization is found. Steiner (9) says that "sometimes specialization may reach such a degree that finally even new hosts of the closest taxonomical, physiological, and chemical relationship to the old host are attacked no more or very lightly." However, biological strains exist that may have a wide host range.

Goodey (5) presented evidence to support the existence of biological strains of nematodes in the species *Ascaris lumbricoides*, *Ditylenchus dipsaci* and *Heterodera schachtii*. He referred to the results of different investigators

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that indicated the existence of a polyphagous race of *D. dipsaci* that attacks potatoes and pasture plants; a phlox race that was not highly specialized; a red clover race that would not attack oats, white clover or lucerne; a white clover race that would not attack red clover; and two strains from narcissus that would not attack oats or red clover. He also quoted experiments that suggested that the narcissus and hyacinth nematodes in Holland were biologically different.

Godfrey and Scott (4) recently observed *D. dipsaci* on salsify, parsley, and celery, and by cross inoculations succeeded in inducing infestation of these hosts with nematodes from garlic and parsley but not from alfalfa. They concluded that the salsify, parsley, celery and garlic nematodes were identical, but the alfalfa nematodes were distinct biologically.

The narcissus nematodes in America are apparently quite polyphagous, for Cobb, Steiner and Blanton (3) observed that they entered 29 hosts in the presence of the population host (narcissus). Courtney (1) has transferred the narcissus nematodes to beans, peas, spinach, oats, and vetch; and Hastings, Bosher, and Newton (6) have transferred them to barley, oats, and wheat.

Experimental

Transfer of Narcissus Nematodes to Other Hosts by Leaf Inoculation

The narcissus strain inoculum was secured from narcissus bulbs that had carried infestation for at least four years. The congelations or masses of coiled dormant nematodes from the base of the bulbs were used. The nematodes were placed in direct contact with the leaves by clamping glass rings filled with nematodes and moist peat to the leaves. The nematodes were first activated by suspending them in water before mixing them with the

TABLE I
TRANSFER OF NARCISSUS NEMATODES TO OTHER HOSTS BY LEAF INOCULATIONS

Hosts	No. of leaf sections inoculated	No. of leaf sections with internal nematodes	Hosts	No. of leaf sections inoculated	No. of leaf sections with internal nematodes
Alfalfa	4	0	Orchard grass	12	1
Alsike clover	12	1	Peas	5	0
Awnless brome grass	12	1	Potatoes	13	2
Berseem	2	0	Radish	4	0
Bulbous iris	12	1	Raspberry	5	0
Cabbage	4	0	Red clover	17	3
Carrot	3	0	Reed canary grass	12	1
Common vetch	4	0	Seredella	12	1
Cowpea	39	12	Spinach	29	17
Crested wheat grass	3	0	Sugar beet	15	4
<i>Datura stramonium</i>	15	3	Timothy grass	3	0
Hubam clover	14	2	Tobacco	4	0
Lespedeza	12	1	Tomato	59	34
Lettuce	46	13	White clover	10	2

peat. The inoculated leaf area within the ring was removed at the end of 24 hours, cleared by heating in lacto-phenol and acid fuchsin, and examined under the microscope.

Inconsistent results were obtained, hence the figures are not representative of the susceptibility of the plant involved. The irregular transfers were due to difficulty in maintaining the nematodes in an active state within the confined space of the closed glass ring. Previous studies (7) have shown that the bulb nematodes lose their motility in the absence of fresh air, or in the presence of decaying matter in the medium. After confinement on the leaf in a glass ring filled with moist sand for 24 hr., all the nematodes were inactive when the ring was removed from the leaves, but when the same nematodes were exposed to air in shallow dishes, some recovered their motility. Although moist peat in the rings was found more effective than sand, nevertheless the motility of the nematodes was preserved with difficulty.

Transfer of Narcissus Nematodes to Other Hosts by Soil Inoculation

Steam sterilized soil was inoculated with water suspensions of narcissus nematodes. Various seeds were sown, and a few days after the seedlings appeared above ground they were cut off close to the soil and prepared for microscopic examination by the lacto-phenol method. Owing to difficulty in handling the seedlings after heating in lacto-phenol, they were covered with the solution and heated on the glass slides.

TABLE II
TRANSFER OF NARCISSUS NEMATODES TO OTHER HOSTS BY SOIL INOCULATION

Hosts	No. of seedlings examined	No. of seedlings with internal nematodes	Hosts	No. of seedlings examined	No. of seedlings with internal nematodes
Alfalfa	6	1	Sainfoin	5	0
Alsike clover	30	3	Siberian millet	15	3
Barley	13	3	Shabdar	35	1
Berseem	10	1	Spinach	6	1
Carrot	5	1	Strawberry	15	4
Cauliflower	9	2	Sweet clover	50	3
Common millet	20	2	Sweet pea	3	0
Japanese millet	20	0	Timothy grass	20	0
Lespedeza	12	0	Tobacco	19	1
Pea	7	2	Tomato	10	3
Reed canary grass	6	0	White clover	5	2
Red clover	17	5			

The nematodes usually entered the seedlings at the crown, in the stem just below it, or in the leaf petioles. Occasionally they entered the leaf blade. Affected red clover seedlings showed a very slight crown enlargement, but the leaf blades and petioles of second and third leaves did not develop per-

ceptible symptoms of infestation. Peas were attacked in the stem and leaf bracts, where white spots appeared, and infestation usually stunted the plants. The appearance of characteristic white spots on barley has been described in a previous publication (6).

The narcissus strain entered 29 hosts in the two experiments.

Transfer of Red Clover Nematodes to Other Hosts by Soil Inoculation

Infested red clover plants were grown in steam sterilized soil. After about six months the nematodes were abundant throughout the soil mass. To create plenty of inoculum the plants, when removed, were ground and mixed with the soil. Various seeds were sown and the seedlings were prepared for microscopic examination by the lacto-phenol method soon after they appeared above ground.

TABLE III
TRANSFER OF RED CLOVER NEMATODES TO OTHER HOSTS BY SOIL INOCULATION

Hosts	No. of seedlings examined	No. of seedlings with internal nematodes	Hosts	No. of seedlings examined	No. of seedlings with internal nematodes
Alfalfa	15	5	Peas	5	0
Alsike clover	15	3	Red clover	18	5
Awnless brome grass	10	0	Reed canary grass	20	0
Barley	6	2	Siberian millet	13	0
Berseem	21	8	Spinach	4	0
Carrot	7	1	Strawberry	16	3
Common vetch	8	2	Sugar beet	4	2
Hungarian millet	5	0	Sweet clover	15	1
Japanese millet	10	0	Tomato	10	3
Kale	4	0	White clover	17	7
Lespedeza	5	0			

The red clover nematode is evidently polyphagous. Among the crops entered, white clover and alfalfa deserve special mention, since others have reported that the red clover strain does not attack these crops. The infestation of strawberry is of interest, for a natural transfer from red clover to strawberry in Washington State was reported by Courtney (2). Infested red clover seedlings in these experiments developed pathological symptoms. At first a pronounced swelling of the crown appeared and occasionally a bend. As the seedlings grew older, the petioles of the leaves frequently became wrinkled, enlarged and deformed, and the plants were conspicuously stunted. The white spots characteristic of the red clover strain on barley were similar to those induced by the narcissus and iris strains on barley. The nematodes entered most of the seedlings near the crown.

Transfer of Strawberry Nematode to Other Hosts by Soil Inoculation

A single infested strawberry plant was grown in steam sterilized soil. Around the infested plant, strawberry seeds were planted. When the seedlings appeared, their enlarged crowns proved that the soil had become in-

festated. The original plant and the infested seedlings were removed, pulverized, and returned to the soil. Various seeds were sown and the seedlings were examined soon after they appeared above ground.

TABLE IV
TRANSFER OF STRAWBERRY NEMATODES BY SOIL INOCULATION

Hosts	No. of seedlings examined	No. of seedlings with internal nematodes	Hosts	No. of seedlings examined	No. of seedlings with internal nematodes
Alfalfa	10	0	Seredella	18	0
Alsike clover	34	0	Spinach	7	1
Barley	10	0	Strawberry	6	2
Carrot	20	0	Sugar beet	6	0
Common vetch	8	0	Sweet clover	19	2
Cauliflower	4	1	Tomato	14	0
Peas	4	0	White clover	22	0
Red clover	15	3			

The strawberry strain infested fewer hosts than the other strains under study. Although the strawberry strain entered red clover, no apparent symptom or pathological effects comparable to those produced by the red clover strain were found.

Transfer of Iris Nematodes to Other Hosts by Soil Inoculation

Severely infested Iris "Supreme" bulbs were obtained from local stock known to have been infested for three successive years. The bulbs were crushed in water and the nematode suspensions were washed once in a weak Cheshunt solution, and introduced into autoclaved soil. The seedlings planted therein were examined as previously described, as soon as they appeared above ground.

The host range of the iris nematode appears to be wide. As in the case of the narcissus and strawberry strains the iris nematode entered red clover but did not induce swelling of the crown or other visible pathological symptoms.

TABLE V
TRANSFER OF IRIS NEMATODES TO OTHER HOSTS BY SOIL INOCULATION

Hosts	No. of seedlings examined	No. of seedlings with internal nematodes	Hosts	No. of seedlings examined	No. of seedlings with internal nematodes
Alfalfa	11	1	Spinach	4	0
Alsike clover	30	0	Strawberry	8	0
Barley	27	2	Sugar beet	5	1
Carrot	23	3	Tomato	10	2
Cauliflower	5	1	White clover	16	6
Red clover	15	3			

Discussion

The establishment of biological forms, races or strains of parasitic fungi by differential reactions on distinct hosts has been of great value to agriculture. Breeding for rust- and smut-resistant wheat can now proceed with the reasonable certainty that a new creation will be resistant to the forms present in the country where the new varieties are to be grown. In the case of the bulb nematode, *D. dipsaci*, much work has yet to be done before the identity and relationships of the biological strains are properly known. This investigation establishes the existence of three strains of *D. dipsaci* in the Pacific Northwest, *viz.*:

(1) Red clover, characterized by causing swollen crowns and stunt in red clover seedlings.

(2) Strawberry, characterized by a limited host range and swollen crowns in strawberries; entrance, but absence of symptoms, in red clover seedlings.

(3) Narcissus and iris, characterized by a wide host range; entrance, but absence of symptoms, in red clover and strawberry.

The red clover strain of the Pacific Northwest may be distinct from the form that exists in Great Britain. Goodey (5) reported that the red clover strain would not attack alfalfa and white clover. The form studied by us entered both these hosts.

It is unlikely that the narcissus strain studied by us is distinct from the European form owing to the quantity of European stock that is planted annually in British Columbia. Hodson (8) was unable to induce the narcissus form to attack oats and red clover, but since symptoms rarely appear in oats and have never been observed by us in red clover, the presence of the nematode in these crops may have been missed although it may be easily established by the lacto-phenol acid fuchsin technique.

The results presented herein are essentially preliminary. We have not yet developed a satisfactory technique for establishing the host range of the biological strains of *D. dipsaci*. The clamping of glass rings filled with a nematode suspension in moist pulverized peat to the foliage of test plants did not affect the plants in a constant manner. The examination of seedlings after clarification in lacto-phenol acid fuchsin solution gave more constant results. The seedlings were removed from infested soils shortly after they appeared above ground.

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THE NEMATODE DISEASE OF BULBOUS IRIS CAUSED BY *DITYLENCHUS DIPSACI* (KÜHN 1858) FILIPJEV 1936, AND EXPERIMENTS ON ITS CONTROL BY BULB TREATMENT¹

By WILLIAM NEWTON², R. J. HASTINGS³ AND J. E. BOSHER³

Abstract

The individual bulbs that arise from a nematode-infested mother bulb are seldom all infested, which is accounted for by the disappearance of the old bulb, when the daughter bulbs form, necessitating a re-entrance. The nematodes appear to attack the stem and tunics first. Normally they enter the bulb proper through the base. Tip infection occurs, but is comparatively rare. No evidence has been obtained that nematodes enter the plants above soil level.

Basal discoloration, as revealed by the removal of the dry caps from the bulb bases, is suggested as a diagnostic symptom in addition to the characteristic streaks in the outer fleshy scale and the discoloration at the base of the stems.

Death and chlorosis of iris plants could not be attributed to bulb nematodes in infested plantations.

No evidence was obtained that the bulb nematode significantly affects the forcing capabilities of iris bulbs.

Immersion in water for one hour at 44° C. killed iris bulbs when done early in November, with or without disinfectants. Treatments of iris bulbs with cold organic mercury solutions and other solutions, increased the yield of bulbs, apparently because they controlled *Penicillium* sp. and other parasites rather than bulb nematodes. Fumigation with ethylene dichloride and ethyl acetate injured iris bulbs and failed to control the nematodes. Formalin as a fumigant was less injurious, but it was not effective as a nematocide.

Introduction

Bulbous irises have been known as hosts of *Ditylenchus dipsaci* since 1925, but little progress has been made in the study of the disease and its control. Apparently the disease has attracted little attention, for Hodson (3) states that not until 1932 was its occurrence in England noted in the English iris (*I. xiphiodes*) and in 1933 in the Dutch iris (*I. xiphium hybridum*).

In America the disease was noted in 1928 and Steiner and Buhner (5) published a summary of their observations between 1928 and 1932, reporting that 22 varieties of bulbous irises were infested, including Spanish (*I. xiphium* L.), Dutch (*I. xiphium hybridum*), English (*I. xiphiodes* Ehrb.) and Moroccan (*I. tingitana* Boiss and Rent) irises. In British Columbia, we noted the disease in 1934 (2). Since then, infestations have been found in many varieties of the Dutch iris, including Wedgewood, Imperator, Yellow Queen, Hart Nibbrig, White Excelsior, and Supreme. During the last two years, a large

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proportion of the foreign iris bulbs examined at the port of entry to British Columbia, were found to be infested. Apparently the infestation in iris is widely spread throughout the world.

Goodey (1) and Hodson (3) have sounded warnings of the increasing incidence of the disease in England. Hodson realized the danger of *D. dipsaci* to the iris bulb and published a review of his preliminary experiments and observations in 1934, in which he described the bulb and field symptoms.

The Iris Bulb in Relation to the Nematode Disease

In the multiplication of bulbous irises, the original bulb breaks down to provide nourishment for the daughter bulbs, hence a nematode infestation in irises is never more than one year old. In narcissus, on the other hand, the bulb tissue is perennial, hence a nematode infestation may be several years old. The fleshy scales of the parent iris bulb shrivel up and decay, hence when they are infested the nematodes are discharged into the soil. These nematodes may re-infest the daughter bulbs. An iris plant is composed of a single stem attached to the basal plate of the mother bulb. This plate bears a cluster of five bulbs usually. These bulbs are arranged fanwise, the largest in the centre, and the stem is next to the central bulb. At maturity the base of the stem is often infested with nematodes. The affected stem may or may not show definite streak symptoms (Fig. 1, 3). The gray or yellow discoloration of the stem may continue into the basal cap (plate of the mother bulb) and into the basal plate of one or more daughter bulbs. From the basal plate of the daughter bulbs the nematodes work up into the fleshy scales, producing lesions that are visible as gray or yellow streaks. The central large bulb of a cluster is often the only one that is infested as the natural consequence of its position next to the infested stem.

Sometimes the nematodes attack the tunic of the bulb, but they apparently exist but a short time in an active state upon the tunics. The nematodes on the tunics are usually found in their characteristic coiled inactive state. Dr. G. Steiner, Senior Nematologist of the U.S. Department of Agriculture, estimated the presence of approximately 3000 nematodes in the tunic of a single bulb forwarded from this laboratory. After a rain, when the bulbs are moist, the nematodes appear to travel in the water film, and thus they gain entrance to the tip or nose of the bulb and basal plate. No evidence has been obtained that the nematodes ever enter the plant above the soil level. The existence of nematode populations in the basal parts of the stem, in the residual parts of the mother bulb and in the tunics, emphasizes the need of special precautions in the destruction of iris bulb cleanings, which are composed chiefly of these plant parts.

Diagnosis of Infestation

A. Infestation with Definite Symptoms

When an infestation originates in the base of the bulbs, the nematodes may produce internal lesions in the outer fleshy scale. Occasionally the second scale is also attacked. The infestation is normally so close to the outer

surface that it may be seen beneath the cuticle as gray or yellow streaks (Fig. 1, 2). Occasionally the lesions are nearer the inner surface, then the infested bulb appears healthy externally, but if the scale is removed, the streaks will be found on the inner surface (Fig. 1, 4).

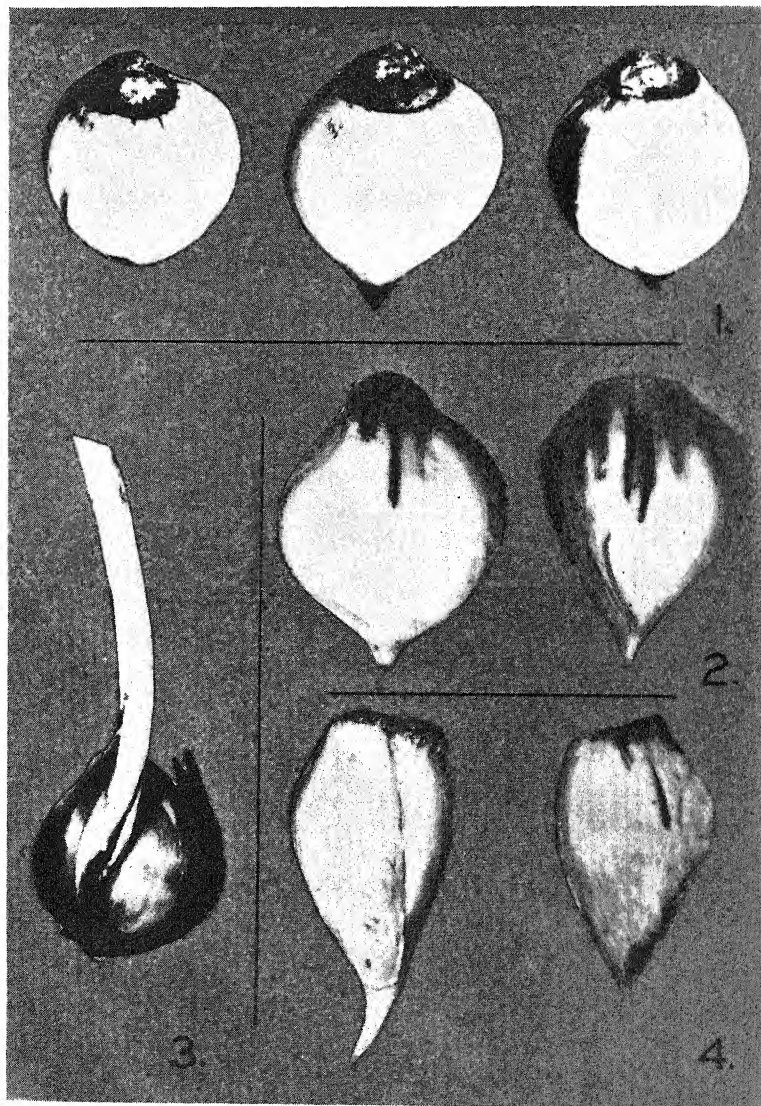


FIG. 1. 1. The characteristic basal discoloration and shrinkage of the tissue around the basal plate of iris bulbs caused by the bulb nematode. 2. The characteristic streaks radiating from the basal plate on the outer surface of the fleshy scale of Wedgewood bulbs. 3. The characteristic streak symptoms on the stem of an iris plant caused by the bulb nematode. 4. The characteristic streak symptoms on the inner surface of the first fleshy scale of a Wedgewood bulb.

B. Infestation with Less Definite Symptoms

Frequently an infested bulb bears no streak symptoms yet may be discolored at the base beneath the dry cap. This discolored tissue around the base may shrink to form a depressed ring which is usually filled with dust-like nematode debris (Fig. 1, 1). An examination of the discolored tissue of the ring, or beneath the cap of the mother bulb, will reveal living nematodes. A quantity of Wedgewood bulbs was examined, and 65% of the infested bulbs exhibited this basal discoloration, while only about 5% bore the characteristic streak symptoms. In one lot of Imperator bulbs, 60% of the bases were found to be discolored when the residual plates of the mother bulbs were removed, yet only a small proportion bore in addition the characteristic streak symptoms. These bulbs were kept in storage for ten weeks, but no further development of streak symptoms occurred. Whether the bulbs show streak symptoms or not, apparently discoloration of the base is a characteristic symptom of infestation.

Suggested Method of Examination for Nematode Infestation

A. Field Examination

Dig up plants at random, remove the small bulbs from the side of the cluster and expose the stem. If the stem is discolored keep it for laboratory examination. Examine the base of the bulbs by removing the basal cap (residual plate of mother bulb) and if any are discolored, proceed to peel back the tunics to expose characteristic streak symptoms. Streak symptoms may be considered as positive evidence of a nematode infestation. When no streak symptoms are present, tease out the discolored stem and basal tissue in water and search for living specimens of *D. dipsaci* under a binocular microscope.

B. Examination of Bulbs after Harvest

When basal parts of the stem still adhere to the bulbs, discoloration thereon is an index of an infestation, but the cleaning process usually removes all stem parts. Proceed with the examination by removing the residual parts of the mother bulb from the base and look beneath for discolored tissue, which may or may not extend upwards as streaks in the fleshy scales. When streaks are not present, a microscopic examination of the discolored basal tissue should be made to certify that the discolored base ring symptom is due to nematodes.

Hodson (3) has suggested that a field infestation is quite obvious, the foliage being chlorotic, dwarfed, or even entirely absent. No case has been found by us where blank areas in iris plantations could be attributed to a nematode infestation. All the dead plants in a heavily infested plantation of Imperator iris were examined. Only 13% were found infested with *D. dipsaci*. On the other hand, Penicillium rot appeared to account for the death of 93%. In a nematode infested plantation of Wedgewood iris, no nematodes were found in the dead plants, and 58% of the dead plants were in an advanced state of

decay through *Penicillium* rot. Chlorotic plants were also examined, but again *Penicillium* rot rather than bulb nematodes appeared to account for the chlorotic symptoms when virus disease was not present.

Effect of Infestation on Bulb Crop

Twenty-four nematode infested bulbs were planted in a glasshouse and the growth compared with 24 healthy ones of corresponding sizes. During the growing season there was no visible difference between the healthy and the infested. At maturity, the 24 infested yielded 55 daughter bulbs that weighed 94 gm., and the 24 healthy yielded 62 that weighed 78 gm. Only 7.2% of the daughter bulbs from infested parents were infested, and in these the bulb nematode was found only in the tunics. No streaks or discolored base tissue were found.

Effect of Infestation on Flower Crop

Data on the percentage of flowers obtained from forcing infested iris bulbs were obtained through the co-operation of Mr. H. F. Olds, District Officer, Plant Quarantine Office, Vancouver, British Columbia, and two Vancouver glasshouse operators. One forcer planted 15,000 Wedgewood bulbs known to have a 5% infestation. A normal harvest of bloom was obtained. From 85 to 95% of the bulbs yielded marketable bloom. An examination of the plants after forcing showed that 23% were infested with *D. dipsaci*. The other forcer planted 6000 Wedgewood bulbs known to have a 25% infestation, and he likewise cut a normal crop, for 90 to 95% of the bulbs yielded marketable bloom. This study suggests that the bulb nematode does not significantly affect the forcing capabilities of iris bulbs.

Control Experiments

Imperator bulbs were used in this experiment, 20% of which bore conspicuous symptoms of nematode infestation. The bulbs were divided into lots of 1000 gm. each and treated thermally, chemically, and by fumigation between November 5 and 7, and planted almost immediately. The notes in Table I were taken the following summer when the plants matured. The bulbs that were treated with hot water failed to grow, possibly because a thermal treatment in early November is too late. Hodson (3) likewise found that a one- and two-hour immersion in water at 110° F. in December destroyed the bulbs. On the other hand, Staniland (4) found that bulbs of the variety Imperator successfully withstood treatment for 50 minutes at 110° F. in early autumn. These discrepancies may be accounted for by the time of treatment. Evidence is accumulating that a one- to two-hour immersion at 110° F. may not injure iris bulbs significantly, if applied shortly after the bulbs mature, but late treatments are unquestionably injurious. According to information received from Dr. Freeman Weiss of the U.S. Department of Agriculture, a modified treatment has been developed which consists of the immersion for 2½ hr. at 70 to 80° F., followed immediately by immersion at 110° F. for 1 hr.

TABLE I
THE INFLUENCE OF BULB TREATMENTS UPON NEMATODE INFESTED IRIS

Treatment				Weight of bulbs at harvest, gm.	Plants infested, %	Flower produc- tion, %
Solution	Concen- tration, %	Period, hr.	Tempera- ture, °C.			
<i>Cold chemical treatments</i>						
Silver nitrate	0.05	1.0		810	34	57
Potassium cyanide	.15					
Formalin	.5	1.0		1540	41	69
Sodium bisulphite	.25	1.0		1317	38	65
Phenol	.25	1.0		1610	62	75
Leytosan (mercurial)	.03	1.0		1790	46	78
Ethyl acetate	1.0	18.0		90	23	—
Cupric nitrate	.25	1.0		940	30	56
Potassium cyanide	.25					
<i>Fumigation</i>						
Ethylene dichloride	.03	24.0		490	15	37
Ethyl acetate	.03	24.0		238	28	38
Formaldehyde		24.0		1172	36	59
Formalin	.01					
KMnO ₄	.01					
<i>Thermal treatments</i>						
Silver nitrate	0.05	.25	50	0		
Potassium cyanide	.15	.5	47	0		
		1.0	44	0		
Formalin	0.5	.25	50	0		
		.5	47	0		
		1.0	44			
Water		.25	50	0		
		.5	47	0		
		1.0	44	0		
<i>Control</i>						
Untreated				1220	56	70
Untreated				1485	55	84

This treatment has apparently done no significant injury to the bulbs when applied not later than early October. There may be a safe period for the immersion of iris bulbs in hot water for 1 hr., but this period depends upon the environmental complex controlling growth, hence it should be determined for each locality.

The cold chemical dips failed to eradicate the bulb nematodes. A mercurial dip treatment gave the highest yield, followed by phenol and formalin.

The increased yield following mercurial and other dips is due apparently to the control of *Penicillium* sp. and other parasites rather than bulb nematodes. The silver-nitrate-potassium-cyanide and cupric-nitrate-potassium-cyanide solutions lowered the vitality of the plants. The toxic effect of ethyl acetate as a dip and as a fumigant was particularly evident. Ethylene dichloride likewise was toxic to iris bulbs. Formaldehyde as a gas was the least injurious, but failed to eradicate the nematodes. No evidence was obtained that the bulb nematode can be controlled by either a chemical dip or by fumigation.

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THE TREATMENT OF GLASSHOUSE SOILS WITH CHLOROPICRIN FOR THE CONTROL OF *HETERODERA MARIONI* (CORNU) GOODEY, AND OTHER SOIL PATHOGENS¹

BY WILLIAM NEWTON², J. E. BOSHER³, AND R. J. HASTINGS³

Abstract

Chloropicrin in 1 cc. doses is lethal to bulb nematodes at six inches from the point of injection or within a soil volume of one cubic foot. It is also lethal to fungi. The vegetative stages are destroyed at 1 : 195,000, and certain sclerotia at 1 : 90,000.

The injection of chloropicrin into greenhouse soils lowered the incidence and pathogenicity of a root-knot infection on both a summer and winter crop of tomatoes and greatly increased the yields of fruit. The chloropicrin costs \$1.65 per pound or \$8.50 to treat 2000 sq. ft. of soil.

Introduction

The artificial conditions of crop production under glass often result in an accumulation of soil-inhabiting pathogens. The most satisfactory method of destroying these is the steam grid method of soil sterilization, but where steam is not available, the operator is left with the alternative of replacing the original soil with uninfested soil, or of treatment with chemicals. The removal and replacement of glasshouse soils is not favored by glasshouse operators. Apart from the expense, the operator is naturally reluctant to remove soil which has been abundantly supplied with plant nutrients by fertilizer applications.

Many chemicals have been used in the past for treating glasshouse soils, but few possess the properties of an insecticide, fungicide and nematocide. Recent researches have suggested that chloropicrin possesses all three properties.

Godfrey and his associates have done much to prove the value of chloropicrin as a soil sterilizer through their studies on the control of *Heterodera marioni* in the pineapple plantations of Hawaii. Johnson and Godfrey (5) found that chloropicrin was an effective nematocide when introduced in liquid form into holes 5-6 in. deep, spaced 18 in. apart, and covered immediately with mulch paper, when applied at the rate of 163 lb. or more per acre. Godfrey (1) subsequently compared chloropicrin with carbon disulphide in pineapple fields and found that chloropicrin applied at 170 lb. per acre reduced the nematode population 90% and increased the yields of pineapple 52%. Carbon disulphide applied at 750 lb. per acre only reduced the nematode population 48% and increased pineapple yields 29%.

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Godfrey (2) and Godfrey, Oliveira, and Hoshino (4) have also shown that the efficiency of chloropicrin can be increased by better confinement of the gas. In some of their tests a 99% control of *H. marioni* was obtained. They considered the following conditions as essential in an efficient control; the application of 250 to 400 lb. per acre; a loose soil without excessive moisture; the injection of the chloropicrin into holes not more than 18 in. apart; and a soil cover of material impervious to gas to prevent atmospheric loss.

The fungicidal properties of chloropicrin have also been investigated by Godfrey (3). He found that 1½ cc. of chloropicrin in a four-gallon jar of soil (400 lb. per acre) sealed with glue-coated cover, destroyed *Fusarium* sp. from gladiolus; *Verticillium albo-atrum* from strawberry; *Phytophthora cactorum* from snapdragon; *Rhizoctonia Solani* and *Sclerotium Rolfsii* from sugar beet; *Armillaria mellea* and *Dematophora* sp.

Experimental

An arrangement was made to conduct an experiment in the root-knot infested bench soils of two local commercial glasshouses each 80 by 25 ft.

The Diffusion of Chloropicrin in Loam Soil

Preliminary to the large-scale glasshouse test, laboratory experiments were conducted to ascertain the relation of the spacing of soil injections to the amount of chloropicrin used in each injection. The lethal effect upon *Ditylenchus dipsaci* (Kühn 1858) Filipjev 1936, the bulb nematode, was used as the measure of efficiency. Congelations of the pre-adult stage of this nematode served as convenient inoculum, for the direct observation of their motility or non-motility leaves no doubt as to the lethal effect of the gas.

The nematode congelations were placed in small glass vials (4 mm. × 2.5 cm.) arranged 2 in. apart on a cardboard strip. A trench was dug in a loam soil to a depth of 8 in., and the cardboard was laid in place. Chloropicrin was applied at one end of the series and the soil returned to the trench. At the end of seven days, the vials were dug up and the nematodes were shaken out into shallow dishes containing tap water. They were left for 24 hr. to recover their motility, and were then examined under a microscope.

TABLE I

THE MOTILITY OF *D. dipsaci* AS AFFECTED BY THE SPACING OF SOIL INJECTIONS AND THE AMOUNT OF CHLOROPICRIN PER INJECTION

Amount of chloropicrin applied (cc.)	Per cent motile							
	Distance in inches from source of gas							
	2	4	6	8	10	12	14	Control
1.00	0	0	0	25	60	90	90	90
2.00	0	0	0	25	40	90	90	90
4.00	0	0	0	4	8	90	90	90
6.00	0	0	0	2	12	90	90	90

These preliminary tests indicate that the maximum distance between injections, where no impervious paper covering is used, should not exceed 12 in., and that there is little difference in efficiency between small and large injections except when the distance between them is more than 12 in.

Preliminary tests were also made to ascertain the fungicidal properties of chloropicrin towards a few common organisms. A concentration of 1 : 195,000 destroyed the vegetative stage of the following fungi: *Fusarium conglutinans* var. *Callistephi*, *F. bulbigenum*, *F. graminearum*, *F. culmorum*, *Phomopsis* sp., *Botrytis Tulipae*, and *Sclerotium Delphinii* (from tulip). The sclerotia of *Rhizoctonia Solani*, *Sclerotinia sclerotiorum*, and *Botrytis Tulipae* were killed at a concentration of 1 : 90,000, but the sclerotia of *Botrytis narcissicola* and *Sclerotium Delphinii* survived, though their germination was retarded more than two weeks as compared with that of untreated sclerotia.

These results show that chloropicrin is extremely toxic to growing mycelia but that resting sclerotia are somewhat more resistant. The experiments were conducted in a 39-litre air-tight vessel, wherein as little as 0.2 cc. of chloropicrin destroyed the mycelial growth of agar cultures. This is a much lower concentration than that used by Godfrey, but confirms his conclusion that chloropicrin is a potent fungicide.

Glasshouse Experiments

Four benches, each 80 by 4 ft. were placed at our disposal by a grower, and the following treatments were made:

- (1) Chloropicrin was injected into holes 6 in. deep, 1 cc. per sq. ft.; the soil was not covered with gas-impervious material.
- (2) Field peas were sown as a trap crop and two weeks later chloropicrin was injected.
- (3) Calcium cyanamide was applied at the rate of 1000 lb. per acre.

Each bench was divided into four plots to carry the three treatments and a control. The chemicals were applied in October, and a winter crop of tomatoes was grown. The last fruit was harvested on February 22.

Owing to unfavorable conditions for pollination, the yields were low. The plants from untreated plots averaged 8.4 oz. of fruit, those treated with calcium cyanamide 10.3 oz. and those from the chloropicrin-treated plots 11.5 oz. Up to the first ten weeks, the best growth was in the calcium cyanamide plots, but after 15 weeks, the chloropicrin plots were the best. Chloropicrin appeared to induce a better root system. This may account for the gains as the season advanced.

Although the tomato plants had splendid root systems on the chloropicrin plots, they were not entirely free from root-knot infestation, but the knots were few in number and small in size, and the infestation did not appear to affect the thrift of the plants. On the other hand, the plants from the calcium cyanamide and untreated plots were badly infested with root-knot, were deformed, and carried few fibrous roots.

TABLE II

THE AVERAGE YIELD OF WINTER TOMATOES AS INFLUENCED BY TREATING ROOT-KNOT INFESTED SOIL WITH CHLOROPICRIN AND CALCIUM CYANAMIDE

Bench No.	Average yield, oz. per plant			
	Chloropicrin treatment, 1 cc. : 1 sq. ft.	Pea trap crop and chloropicrin treatment	Calcium cyanamide, 1000 lb. per acre	Control
1 (west)	15.3	14.9	11.4	7.5
2 (middle)	9.4	10.7	10.0	9.4
3 (middle)	10.9	11.6	10.0	5.9
4 (east)	10.7	10.5	9.7	10.4
Average	11.5	11.9	10.3	8.4

The experiment was repeated in the spring with the summer crop of tomatoes.

All the plots were dug and trenched with straw, and the chemicals were applied in February, but the pea trap test was abandoned and replaced by a cupric potassium cyanide drench. The solution was prepared by dissolving 0.25 lb. of cupric nitrate and 0.25 lb. of potassium cyanide in 10 gallons of water, and one gallon was applied to each square foot of soil.

The yield from this crop was satisfactory. The average weight of fruit per plant is shown in Table III.

TABLE III

THE AVERAGE YIELD OF SUMMER TOMATOES AS INFLUENCED BY TREATING THE SOIL WITH CHLOROPICRIN, CALCIUM CYANAMIDE, AND CUPRIC-NITRATE-POTASSIUM-CYANIDE

Bench No.	Average yield, oz. per plant			
	Chloropicrin treatment	Cupric-nitrate-potassium-cyanide treatment	Calcium cyanamide treatment	Control
1 (west)	87.3	72.7	73.5	63.9
2	53.4	61.7	54.5	62.5
3	57.8	48.3	49.8	43.9
4 (east)	76.2	54.3	57.2	56.6
Average	68.7	59.2	59.0	56.7

The plant growth on all the plots was good. After the crop was harvested the plants were dug up and examined. Those from the chloropicrin-treated plots were still slightly infested with root-knot, the infestations being confined to the deeper small roots, but the galls were small and few in number. The plants from the control plots and calcium cyanamide plots were severely infested with root-knot. No root-knot was found in the roots from the plots that were treated with cupric-nitrate-potassium-cyanide, but there was evidence of injury.

The yield increase of 12 oz. of fruit per plant after the chloropicrin treatment represents an enhanced crop value of about \$45.00; this calculation is based upon 600 plants and fruit at 10 cents per pound.

As the rate of application was 1 cc. of chloropicrin per square foot, and there are 266 cc. per pound of chloropicrin, the four benches of soil used approximately 5 lb. of gas costing \$8.40 (chloropicrin costs \$1.68 per pound in British Columbia). The net profit from the treatment, therefore, amounts to about \$36, not including its benefit to subsequent crops.

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THE INFLUENCE OF LIPOIDS ON THE QUALITY AND KEEPING PROPERTIES OF FLOUR¹

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Abstract

The keeping properties of different flours varied considerably. Aging was accompanied by increased absorption regardless of whether the flour deteriorated in baking quality. Increased acidity developed in all flours, but was not a good measure of deterioration. Storage in sealed containers favored acidity increases, while storage in sacks favored deterioration. A decrease in ether extract accompanied deterioration, while decrease in the less soluble lipoids appeared to take place in all samples.

The changes in physical properties of gluten gave the best indication of deterioration. Gluten from deteriorated flour was harsh, spongy and short, and could not be completely dispersed in sodium salicylate. Unsaturated fatty acids added to flour had the same effects on gluten, but not on baking quality. Ground wheat germ added to deteriorated flour improved the gluten and restored its solubility. The more insoluble germ lipoids were the effective substances. Alcohol extraction of flour caused deterioration, but gluten quality was largely restored by addition of germ. All results indicate the lipoids are adsorbed on the protein of gluten.

It is concluded that the unique physical properties of gluten are to a considerable extent dependent on the relatively insoluble lipoids present. The possibility of gluten denaturation being a breakdown of the protein-lipoid complex is discussed.

Introduction

The quality and keeping properties of flour milled from wheat grown on the black soil at Edmonton have been found to be superior to those of flour from wheat grown on the gray soil at Fallis (1). The differences in original quality were largely accounted for by differences in protein content, but differences in keeping properties must have been due to other factors. Many of the Fallis samples had deteriorated markedly 10 months after milling, while most of the Edmonton samples maintained their quality for at least 15 months. The keeping properties of the flours milled from different varieties of wheat grown on the same soil differed.

Considerable work has been done on the changes which take place during the aging of flour, but as far as the writers know no results comparable to those obtained with the Edmonton and Fallis flours have been reported. It is known that definite changes in the chemical and physical properties of flour take place during the aging process, and recent work has shown fairly conclusively that the most important changes are in the lipoidal constituents (18).

Since the literature related to this problem has been extensively reviewed (2, 3, 18) no attempt is here made to cover it again. The most extensive and conclusive work related to the present problem permits the following conclusions:

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The changes which take place in an unbleached flour during storage result in improvement in quality until a fairly definite optimum has been reached, after which a gradual or rapid deterioration may set in. It is generally believed that the processes involved in the various stages are the same, deterioration being the result of the processes going beyond the optimum.

The specific substances involved in the changes attendant on flour aging have not been well defined, although phosphatides (8, 20, 21), unsaturated fatty acids (11, 12, 13, 14), oxidized fatty acids (18) and others have been suggested. Older work was based on the assumption that germ, present in the flour as an impurity, was the source of the lipoidal substances involved, but recent investigations (18) indicate that the flour itself is the more important source.

The interpretation of the results presented by various investigators is rendered difficult because there has been no uniformity in the methods used, nor in the terminology employed in discussing the substances studied. The whole class of fat-like substances termed "lipoids" or "lipids" includes compounds of widely varying nature, and the use of various solvents for extraction of these "lipoids" from flour results in the study of varying fractions of the total lipoids. The failure of the investigators to define what is meant by "flour oil", "flour fat" and "germ fat" makes it almost impossible to compare the conclusions reached.

The present paper deals with results of further studies with the material discussed in the previous publication (1), and with results of preliminary experiments designed to clarify some of the points raised in these studies.

Material

The material used in the main part of this study has been briefly described in an earlier paper (1). The 1932 series was milled in the fall of 1932, and baking tests were made 1, 10, 24 and 36 months after milling. The flour was stored in the laboratory in cans with tight-fitting covers. The 1933 series was milled in the fall of 1933 and baked one and 12 months after milling. The flour was stored both in glass sealers with air-tight covers, and in cotton sacks. The moisture content of these flours after 12 months storage was approximately 13% and 8% respectively. Small quantities of wheat of the 1933 series were stored until the spring of 1935, when they were milled, and the flour compared with that milled in 1933.

A high-protein Reward wheat of the 1935 crop was used to provide flour for the other experiments, all of which were carried out within three months of the time of milling. During this time the flour was stored in cans with close-fitting covers.

Protein

Methods

Nitrogen was determined by the Kjeldahl method using mercuric oxide as catalyst. Protein is reported as nitrogen $\times 5.7$, and the results corrected to a 13.5% moisture basis.

Baking

Unless otherwise stated, baking was carried out using the bromate formula with 1 mg. of potassium bromate per loaf. Absorptions are all calculated on the basis of 13.5% moisture, and therefore increases after storage are not due to decreased moisture content of the flour.

Acidity

Acidity was determined by the Greek method (7) using tincture of curcuma as indicator. The results are reported as the number of cc. of *N*/50 sodium hydroxide required to neutralize 10 cc. of the alcoholic extract.

Hydrogen Ion Concentration

The hydrogen ion concentration, expressed in the tables as pH values, was determined using a type K potentiometer with the quinhydrone gold electrode. The determinations were made on suspensions of 10 gm. of flour in 50 cc. of distilled water, the suspension being maintained by constant stirring during the determination.

Ether Extraction

The flour was dried in vacuum over concentrated sulphuric acid until it contained less than 1.5% moisture. A 10 gm. sample was then placed in a centrifuge tube with 50 cc. of diethyl ether, and shaken at frequent intervals for two hours. The contents of the tube were then centrifuged and the ether extract decanted. The extracted flour was washed with two 25-cc. portions of ether and the washings added to the original decantate. Most of the ether was distilled off, the residue placed in a tared vessel and dried to constant weight in an air oven at 60° C.

This simplified technique was found to give results that were in good agreement with those obtained by the usual Soxhlet extraction. Since the Soxhlet extraction could not be used with mixed solvents such as the alcohol-ether discussed in the next paragraph, the simplified procedure was adopted for all flour extractions.

Alcohol-ether Extraction

The extract referred to as the alcohol-ether extract was obtained by a modification of the method described by Channon and Foster (4), and is not comparable to the alcohol-ether extract described by Sullivan and Near (16). This extraction was carried out in a manner similar to that used for ether, except that a 1 : 1 mixture of 95% alcohol and diethyl ether was used as the solvent. In this case, however, the extract, after removal of the ether and alcohol, was re-extracted with hot chloroform. The chloroform solution was placed in a tared vessel, evaporated to a small volume, and then dried to constant weight in an air oven at 100° C.

Gluten Washing

Gluten was washed from the flour by the procedure recommended by Dill and Alsberg (6) except that smaller quantities were used. Washing conditions were standardized as much as possible. For some of the special

studies wash water of various pH values was used with samples of the same flour. The water was made up to the same total salt concentration as that recommended by Dill and Alsberg, the desired pH being obtained by using varying proportions of the phosphates as in Sorensen's buffers.

Baking Tests

Results

A summary of the baking results already discussed briefly by Aamodt and McCalla (1) is presented in Table I. The results for each location and for each year are divided into two groups, representing as closely as possible

TABLE I
MEAN BAKING RESULTS OF 1932 AND 1933 SERIES

Origin	No. of samples	Keeping properties	Absorption, %		Loaf volume, cc.		Partial baking score	
			Fresh*	Stored**	Fresh	Stored	Fresh	Stored
1932								
Edmonton	14	Very good	66	68	696	694	54	54
Edmonton	2	Fair	65	68	744	513	54	52
Fallis	7	Fairly good	68	69	623	594	53	48
Fallis	9	Poor	69	71	613	423	54	37
1933								
Edmonton	8	Good	68	70	712	612	55	54
Edmonton	3	Poor	67	69	723	414	52	43
Fallis	4	Fairly good	66	69	631	532	44	43
Fallis	7	Poor	64	67	587	432	40	36

* Fresh samples baked 1 month after milling.

** Stored samples, 1932, baked 10 months after milling.

Stored samples, 1933, stored in sacks, baked 12 months after milling.

samples behaving alike in regard to keeping properties. There was no sharp dividing line between individual samples, but the differences between these in the two groups of the 1932 series were quite marked. There was distinct variation in behavior of the various samples of the 1933 series, but the division was made after taking all factors into consideration. More extended baking results with the nine Fallis samples of the 1932 series which had the poorest keeping properties and the corresponding nine Edmonton samples are presented in Table II.

TABLE II
MEAN BAKING RESULTS FOR NINE VARIETIES OF THE 1932 SERIES

Time after milling, months	Absorption, %		Loaf volume, cc.		Partial baking score	
	Edmonton	Fallis	Edmonton	Fallis	Edmonton	Fallis
1*	67	69	705	613	55	54
10*	69	71	655	423	55	37
24**	—	—	655	380	65	27
36**	74	75	445	255	44	24

* Average of individual results.

** Composite of the nine varieties.

The higher absorption after storage, recorded in Tables I and II, was apparently independent of deterioration, since the absorption of samples with inferior and satisfactory keeping properties increased almost to the same extent. This conclusion is substantiated by the results for individual samples recorded in Tables III and IV.

The discussion of the baking results as presented in the earlier paper (1) applies to the results in Table I. In 1932 the difference in behavior of the material grown at the two places was more marked than in 1933, but in both years the flour from the wheat grown on the gray soil failed to keep as well in storage as did that from wheat grown on the black soil. Although several of the varieties grown at Fallis produced flour with at least fairly good keeping properties, the only one of commercial importance was Reward.

The two later baking tests on the 1932 samples were carried out after the publication of the earlier paper. The Edmonton-grown material did not deteriorate between 10 and 24 months, but during the same period the deterioration of the Fallis-grown material, which was definite at 10 months, continued. At the end of 36 months, the Edmonton flours had deteriorated only approximately as much as the Fallis flours after 10 months' storage.

Acidity and pH

After the baking tests had been carried out, only small amounts of flour were left. The results of preliminary tests carried out with the 1932 samples are presented in Table III, and the results of similar tests on the 1933 series in Table IV. Baking results are included for purposes of direct comparison with other results obtained.

No analytical studies were made with the flours of the 1932 series at the time of the first baking, but considerable work has shown that acidity and pH values of most freshly milled flours are fairly uniform. The quality of the gluten from Fallis-grown samples is usually only slightly, if at all, inferior to that of corresponding Edmonton-grown samples.

While the acidity of deteriorated flour was, within a variety, higher than for non-deteriorated, the results for the whole 1932 series indicate that acidity is not a reliable measure of either quality or keeping properties. The results with the 1933 series are even more convincing, for analyses were made at the time of the first baking. The apparent discrepancies between the baking and other results of the 1933 series cannot be attributed to the four months' delay in making the other tests, since later baking tests on a short series showed that the relations among samples 12 months after milling had been maintained some months later. The acidities of all samples increased with storage, but the range of increase in samples with similar baking quality was enormous. The I-28-60 sample grown at Fallis and stored in a sealer had the second highest acidity in the series, more than double that of the same sample stored in a sack; but the decrease in loaf volume was just significant, while that of the sample stored in the sack was very marked. Similar results were obtained with other varieties.

TABLE III
RESULTS OF REPRESENTATIVE SAMPLES, 1932 SERIES

Variety	Origin	Protein, %	Absorption, %		Loaf volume, cc.		Acidity, 10 months	pH, 10 months	Gluten quality, 10 months	Moisture content of gluten, %, 10 months
			1 month	10 months	1 month	10 months				
Supreme	Edmonton	14.2	64	65	705	744	1.86	5.60	Excellent	66.5
Supreme	Fallis	11.0	68	69	618	630	1.61	5.70	Good	65.1
Early Triumph	Edmonton	14.4	65	67	702	650	1.26	6.00	Excellent	66.2
Early Triumph	Fallis	10.5	68	70	571	498	2.80	5.70	Poor	62.4
Red Bobs 222	Edmonton	14.2	65	67	632	666	2.18	5.75	Good	64.9
Red Bobs 222	Fallis	10.3	69	70	588	428	3.18	5.65	Very poor	59.7
S-28-2	Edmonton	15.3	62	62	673	484	3.05	5.80	Poor	61.2
S-28-2	Fallis	10.8	64	65	544	503	1.82	5.95	Poor	62.1

TABLE IV
RESULTS FOR REPRESENTATIVE SAMPLES, 1933 SERIES

Variety	Origin	Protein, %	Absorption, %				Loaf volume, cc.				Acidity, cc., 0.02N NaOH				Gluten quality	
			1 month	12 months		1 month	12 months		1 month	16 months		1 month	16 months			
				Sacks	Sealers		Sacks	Sealers		Sacks	Sealers					
Canus Canus	Edmonton Falls	15.1 10.9	65 66	68 68	69 71	697 623	607 446	720 541	1.04 1.10	1.44 1.83	1.41 2.47	Good Good	Good Poor			
Garnet Garnet	Edmonton Falls	14.1 11.7	67 —	69 66	70 70	672 588	457 413	503 498	1.00 —	2.38 1.90	3.57 2.23	Good Fairly good	Poor Poor			
Huron Huron	Edmonton Falls	15.1 10.4	68 66	71 67	73 69	663 558	566 378	623 494	1.20 0.91	1.33 2.37	1.88 2.00	Good Good	Fairly good Poor			
I-28-60 I-28-60	Edmonton Falls	15.0 11.5	68 62	70 67	71 70	792 661	419 519	450 605	1.14 1.04	3.18 1.59	3.49 3.54	Very good Good	Poor Fair			
Red Bobs Red Bobs	Edmonton Falls	14.7 10.6	66 64	69 67	69 69	694 632	627 440	687 602	1.06 1.13	1.69 1.96	1.69 1.55	Very good Very good	Good Poor			

The changes that took place in the two sets of stored flours were considerably different in nature. The increase in acidity was in general favored by the conditions in the sealers, *i.e.*, fairly high moisture content (13%) but no aeration; while the factors more directly concerned with deterioration were apparently favored by aeration in the sacks. Both sets of samples were stored in the same laboratory, so temperature effects should have been similar.

The results reported by Sullivan *et al.* (18) suggest that the smaller amounts of fatty acids liberated in the flours stored in sacks may have been rapidly oxidized, while the larger amounts liberated in some of the flours stored in sealers were not. The amount of acid in the sealer-stored Fallis I-28-60 was equal to 1% of the flour, if all the acid was made up of C₁₈ fatty acids. The effect of 1% freshly mixed unsaturated fatty acids added to flour on the loaf volume of the bread produced was comparatively small, but the effect of the same acids exposed to oxygen for seven days was quite marked (18). This suggests that a small quantity of oxidized fatty acids might be more deleterious to baking quality than a much larger quantity of unoxidized unsaturated fatty acids. The differences in behavior of different samples stored under the same conditions may have been due to differences in the fatty acids present or to differences in the amount and nature of substances which act as catalysts and inhibitors to oxidation.

The pH results for the 1932 series are included in Table III, but they were of less value in indicating deterioration than were acidities. This determination was made on the 1933 series, but the results are not presented since they had no value in appraising either quality or deterioration.

Lipoids

The results of protein and lipid determinations of representative flours of the 1933 series are presented in Table V.

TABLE V
PROTEIN AND LIPOID CONTENT OF FRESH AND STORED FLOURS, 1933 SERIES

Variety	Origin	Protein, %	Ether extract, %		Alcohol-ether extract, %		Ether extract		Alcohol-ether extract	
			Fresh	Stored*	Fresh	Stored*	Protein		Protein	
							Fresh	Stored*	Fresh	Stored*
Huron	Edmonton	15.1	1.10	1.18	1.50	1.44	0.073	0.078	0.099	0.095
Huron	Fallis	10.4	1.18	0.92	1.60	1.34	0.113	0.086	0.154	0.127
Red Bobs	Edmonton	14.7	1.14	1.16	1.60	1.34	0.078	0.079	0.109	0.091
Red Bobs	Fallis	10.6	1.16	0.90	1.74	1.26	0.109	0.085	0.164	0.114
Canus	Fallis	10.9	1.26	0.88	1.54	1.54	0.116	0.081	0.141	0.141
Garnet	Edmonton	14.1	1.10	0.98	1.52	1.36	0.078	0.070	0.108	0.096
I 28-60	Edmonton	15.0	1.16	0.88	1.22	1.00	0.077	0.059	0.081	0.067

* Stored in sacks.

There were only slight differences in the ether extracts of fresh flours, but a decided decrease took place with deterioration. Aging of the flour that did not deteriorate did not, however, appear to affect the amount of ether extract. Various workers have studied the changes that take place in the amount of lipoidal materials during storage (9, 17), but no account of decreases as large as those reported here have been found in the literature.

The effect of storage on the quantity of alcohol-ether extract was not as uniform. Fallis-grown Canus deteriorated, but the amount of this extract remained constant, while Edmonton-grown Red Bobs did not deteriorate, but the extract decreased. The decrease in most of the samples must have been related to the changes that took place in the lipoidal constituents, some of the products of the changes becoming insoluble.

Sullivan and Near (17) have suggested the lipid protein ratio of wheat as an index of quality. Whether the same suggestion might apply to flours is not stated, but in this series the values for fresh flours give little information as to quality not already given by protein values alone. The higher values for the Fallis samples were due principally to the lower protein content, since both the ether and alcohol-ether extracts were only slightly higher than for corresponding Edmonton samples. The decrease in the extract of most of the deteriorated samples reduced the lipid-protein ratio, and should have indicated higher quality in the terms of the Sullivan and Near suggestion. Obviously the suggestion was not meant to cover such a situation, but it is equally obvious that if deterioration entered as a factor in determining quality, the lipid-protein ratio of flour would be unsatisfactory as an index.

The alcohol-ether extracted flour was further extracted with 90% alcohol plus 10% aqueous ammonia at 70° C., using a modification of the method of Sullivan and Near (16) for gluten. Irregular results with poor agreement among replicates were obtained. The solution removed considerable protein from the flour, and re-extraction of the extract with chloroform failed to remove all of the non-lipoidal material. The hot alcohol-ammonia removed as much more lipoidal and accompanying substances from fresh flours as did the original alcohol-ether solution. The extract of stored flour was as much as 50% lower than that of the comparable fresh flour, but deterioration appeared to have little effect on the changes which took place. Despite the fact that the method is not as accurate as the other extraction methods, the results suggest that a decrease in the less soluble lipoidal constituents takes place with storage.

Gluten Quality

The study of the physical properties of gluten as a means of estimating the degree of aging or deterioration in flours has received considerable attention by Kozmin (11, 12, 13) and her co-workers (14). The physical properties of the glutes were directly related to the quality of the flours of both the 1932 and the 1933 series after storage (Tables III and IV), although there were relatively small variations in gluten quality of fresh flours. The changes in gluten quality during storage afforded a good measure of deterioration of

the flours. Fresh and non-deteriorated stored flours yielded glutens with good elasticity and extensibility, while deteriorated flours produced glutens that were harsh, spongy and short. As the moisture content of the glutens decreased (Table III), the quality of the gluten became poorer. Kozmin (12) has concluded that the changes in the glutens during aging are caused by increased concentrations of unsaturated fatty acids. Glutens of the deteriorated type are certainly obtained when such acids are added to flours, but the characteristic decrease in baking quality is not (Table VI). The enormous increase in acidity due to the direct addition of unsaturated fatty acids was only slightly reflected in decreased baking quality. The change in acidity of the Fallis-grown I-28-60 sample during storage had little effect on baking quality, but a definite effect on the gluten. Despite the much smaller increase in acidity in the two other samples listed in Table VI, the effect of aging on both baking and gluten quality was much greater than with I-28-60. Fatty acid accumulation cannot be considered the only, or even the principal, cause of deterioration. This conclusion was also reached by Sullivan *et al.* (18).

The effect of increased acidity of the washing solution on the moisture content of the gluten obtained is shown in Fig. 1. The quality of the glutens obtained at the lower pH values was superior, but the quality of those from deteriorated flours was relatively poor even when the pH was as low as 4.0. Washing at pH 3.5 resulted in dispersion of the gluten from deteriorated flour, while that from non-deteriorated could be recovered in decreased amounts only with difficulty. That the moisture holding capacity of the glutens was related to quality and not to origin is indicated by the two points for Fallis-grown Supreme which fall near those for Edmonton-grown Red Bobs and Early Triumph, and the two points for Edmonton-grown S-28-2 which fall between those for Fallis-grown Red Bobs and Early Triumph. There seems to be no question that as flour deteriorates, the imbibitional capacity of the gluten, under specific conditions, decreases.

Aside from the immediate problem is the fact that the moisture content of the gluten washed from each of the two deteriorated samples increased at pH 7.5, while that of each of the non-deteriorated samples decreased. This suggests some difference in the nature of the glutens in the two types of flour; this problem is being further studied.

Perhaps the best evidence of a definite change in the physical properties of the gluten from deteriorated flour was obtained from solubility studies. Rose and Cook (15) obtained complete dispersion of wet gluten of good quality in 10% sodium salicylate in approximately three hours. The glutens from fresh and undeteriorated stored flours dispersed readily in 10% sodium salicylate and produced viscous, semi-opaque dispersions with no visible particles remaining. The gluten from deteriorated flour could not be completely dispersed, regardless of the length of time used, but broke up into fine particles which settled to the bottom of the comparatively transparent dispersion of low viscosity. Not more than 60% of the protein of some of the samples of gluten was dispersed in five hours. The nature of the portions of the gluten dispersed has not yet been determined.

TABLE VI
BAKING AND GLUTEN QUALITY OF FLOUR AS AFFECTED BY AGING AND THE ADDITION OF FATTY ACIDS

Variety	Time of storage, months	Addition	Acidity, cc. 0.02N NaOH	Total acid,* %	Absorption, %	Loaf volume, cc.	Crumb texture	Gluten quality
I-28-60, Fallis	1	None	1.04	0.29	62	661	6	Good
I-28-60, Fallis	12	None	3.54	1.00	70	605	6	Fair
Red Bobs, Fallis	1	None	1.13	0.32	64	632	6.5	Very good
Red Bobs, Fallis	12	None	1.96	0.55	67	440	3	Poor
Canus, Fallis	1	None	1.10	0.31	66	623	5.5	Good
Canus, Fallis	12	None	1.83	0.52	68	446	4	Poor
Reward	1	None	1.07	0.30	68	802	7	Excellent
Reward	1	1% oleic acid	4.60	1.30	68	760	7	Poor
Reward	1	1% linolic acid	4.48	1.26	68	703	6	Very poor

* Assuming all acid in form of C_{18} molecules.

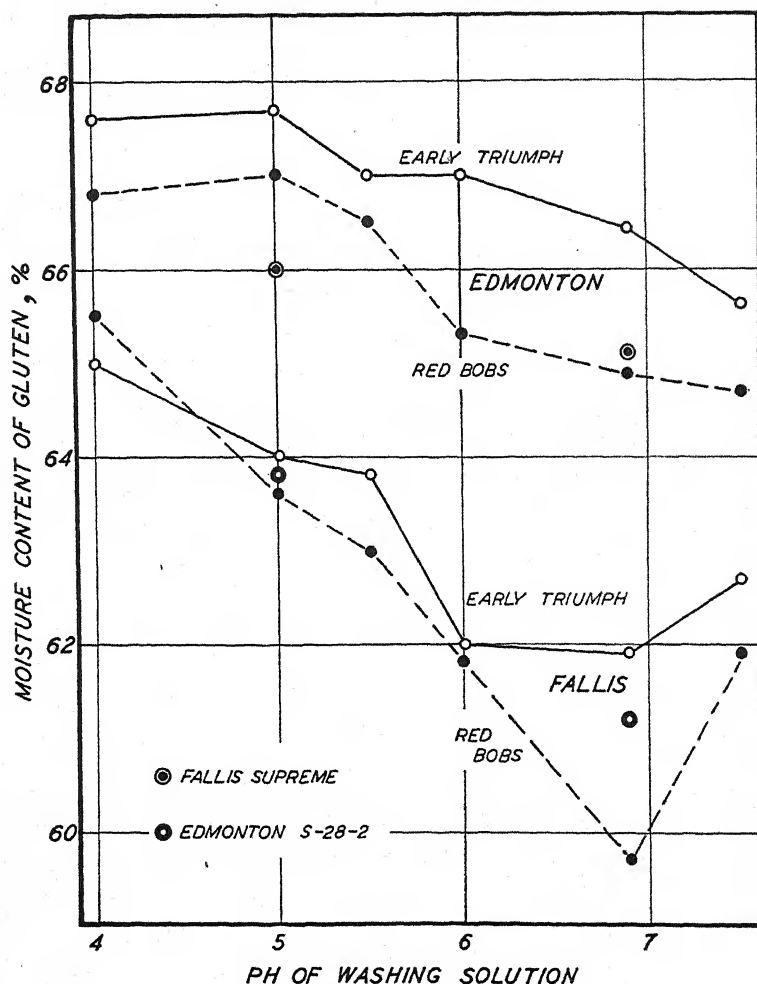


FIG. 1.

Finely ground wheat germ was added at rates of 5 and 10% to the deteriorated flour before the gluten was washed out. The glutes obtained from flours treated in this way were much more extensible and softer, and could be completely dispersed in sodium salicylate. When 10% of the ground germ was added to fresh flour, the gluten obtained could be dispersed in as short a time as 15 minutes.

Extraction of the germ with hot alcohol removed most of the beneficial effects, the gluten from the deteriorated flour and alcohol-extracted germ mixtures being tough, short, and not completely dispersible. Extraction of the germ with ether removed almost 60% of the total lipoidal constituents, but actually increased the beneficial effect of the germ when added to a deteriorated flour. It must be concluded that as it occurs the germ fraction effective in improving gluten quality is insoluble in ether but soluble in hot

alcohol. The improvement resulting from ether extraction was probably directly due to the removal of fatty acids from the germ. Ether extraction of the deteriorated flour itself improved gluten quality, probably for the same reason.

The effects of ether, acetone, alcohol-ether and alcohol extraction of a high grade flour from Reward wheat as indicated by gluten quality are recorded in Table VII. While the glutens from ether- or acetone-extracted flours

TABLE VII

THE EFFECT OF EXTRACTION OF FLOUR BY VARIOUS SOLVENTS, AND ADDITION OF GERM, ON GLUTEN QUALITY

Extraction	Addition	Gluten quality	Dispersion in 10% sodium salicylate
Control	Control	Very good	Complete
Ether	Control	Very good	Complete
Acetone	Control	Good (very firm)	Complete
Alcohol-ether	Control	Poor	Incomplete
Alcohol	Control	Very poor	Incomplete
Control	10% germ	Fairly good (soft)	Complete
Ether	10% germ	Good	Complete
Acetone	10% germ	Good	Complete
Alcohol-ether	10% germ	Fair	Complete
Alcohol	10% germ	Fair	Complete

were of good quality and dispersed completely in sodium salicylate, those from alcohol-ether- and alcohol-extracted flours appeared in every way like glutens from deteriorated flour. Merely subjecting a flour to alcohol wetting, without removing the extract, has a deleterious effect on gluten. It might therefore be argued that the effect of the alcohol extractions was due to alcohol denaturation rather than to removal of the alcohol-soluble lipoids. If this were so, it is difficult to explain why the lipoidal substances added in the form of germ partially restored the other physical properties, and entirely restored the solubility of the gluten.

The effects of fatty acids on some of the physical properties of gluten have already been discussed. Glutens obtained from flours to which oleic or linolic acid had been added could not be completely dispersed in sodium salicylate. The detrimental effects of fatty acids could be largely overcome by adding germ, in addition to the fatty acids, to the flour before washing. All results indicate that lipoidal substances are adsorbed on the protein, and that there is competition in adsorption among the various lipoidal compounds present. The deleterious effect of fatty acids can be overcome by the addition of fairly large quantities of lipoids which improve quality, while the beneficial effects of the latter can be overcome by the addition of relatively large amounts of fatty acids. The difference in effect of fatty acids on baking and gluten quality may be related to this competition effect, the action of the acids being depressed under the conditions of the baking test, but favored under gluten washing conditions.

The effect of germ and extracts on baking quality is somewhat different from the effect on gluten quality, but that germ has a definite improving effect on deteriorated flour is evident (Table VIII). The loaves baked from

TABLE VIII
EFFECT OF GERM AND EXTRACTS ON THE BAKING QUALITY† OF FLOUR

Flour sample	Addition	Loaf volume, cc.	Effect of addition*	Crumb texture
Fresh	Control	760	—	7
Fresh, ether extracted	Control	710	—	7
Aged	Control	480	—	5
Aged, ether extracted	Control	530	—	6.5
Fresh	5% fresh germ	656	-2	5
Fresh, ether extracted	5% fresh germ	500	-5	4
Aged	5% fresh germ	650	+4	5
Aged, ether extracted	5% fresh germ	484	-1	4.5
Fresh	{ Extract from 10 gm. germ**	784	0	6
Fresh, ether extracted		700	0	6
Aged		464	0	5
Aged, ether extracted		556	0	6
Fresh	{ Extract from 100 gm. fresh flour**	816	+1	6.5
Fresh, ether extracted		736	0	7
Aged		536	+1	5.5
Aged, ether extracted		616	+2	6.5

† *Malt-phosphate-bromate formula.*

* *Unit is statistical significant difference = 40 cc.*

** *Germ and flour extracted with alcohol; extract re-extracted with ether. Added in ether solution.*

the fresh and the deteriorated flours to which fresh germ had been added were alike in all respects, the germ having decreased loaf volume and injured the texture of the fresh flour, but markedly increased the loaf volume of the deteriorated flour. Sullivan *et al.* (18) obtained a decrease of nearly 40% in loaf volume by the replacement of 10% of fresh flour by fresh germ, but did not add it to deteriorated flour. They conclude that the deleterious effect of germ is due to some non-lipoidal substance (19). It seems likely, therefore, that in the present experiment, the loaf volume obtained with the two flours is an equilibrium value, the injurious constituents reducing the volume of the bread from the original flour to the same point as the strengthening effect of the other constituents increases the volume of the bread from the deteriorated flour. This improving effect of germ on deteriorated flour has been obtained with a large number of different samples.

The effects of germ on extracted flours are quite different from the effects just discussed. Not only did germ fail to improve the quality of aged flour, but it depressed the quality of the fresh flour to the same extent as did deterioration on aging. No explanation can be offered at the present time.

While the fact that the flour extract was beneficial and the germ extract was not (Table VIII) is in agreement with the results of Sullivan *et al.* (18), the

original quality of the deteriorated flour could not be restored by the addition of such extracts. The effect was definitely increased by ether extraction of the deteriorated flour, and this is also in agreement with Sullivan's results. Two factors of importance must be recognized in the discussion of these differences.

In the first place, deterioration as measured by loaf volume decrease had proceeded much further in the flours used in the present study than in that discussed by Sullivan. There is no question but that much of the disagreement in results obtained in studies such as this may be attributed to inherent differences in the material studied. The reduction in loaf volume of Sullivan's aged sample was only 7% while that of the sample used in the present study was 37%. Ether extraction of Sullivan's sample further decreased loaf volume, but it significantly increased that of our sample. Scattered but extensive studies of ether extraction have led the writers to the conclusion that the extraction of high quality flour, from hard red spring wheat at least, invariably results in decreased loaf volume; but that the extraction of badly deteriorated flour results in improvement. A few instances have been noted where no effect has been obtained with flours which were less severely deteriorated. It seems possible that the flours from different types of wheat might behave differently, and these differences account for such disagreement as reported by Sullivan.

In the second place, the method of preparing the "flour-fat" used by Sullivan is not discussed. The writers have already indicated their belief that the ether-insoluble, alcohol-soluble fraction of the lipoids is responsible for the beneficial effects of germ, and this is extended to include flour also. Efforts to demonstrate this directly have been only partially successful. This fraction cannot be added to flour in alcoholic solution, since the alcohol definitely injures gluten quality. The preparation of the fraction in such form that it can be added without a solvent is very difficult, and so far we have been unsuccessful in preparing an extract that is again completely soluble after the alcohol has been removed. It is probable that in the study here reported the extracts used contained only a small portion of the important lipid fraction. This problem is being further investigated, and is believed to be of vital importance if the role of lipoidal compounds in determining flour and gluten quality is to be definitely established.

Discussion

In spite of the difficulties in devising experiments to prove conclusively that, to a considerable extent, gluten owes its unique physical properties to the presence of lipoids in a complex with the protein, the writers believe this to be so. The various results presented in this paper appear to justify the conclusion that the more insoluble lipoidal constituents of flour are essential for the formation and maintenance of the characteristic dough and gluten structure found with wheat flour. Such a conclusion is neither new nor original. Working (20) in 1924 suggested the possible importance of

lipids in determining gluten quality. The general question of the validity of conclusions regarding the physical properties of proteins based on results obtained with purified preparations has been raised by several investigators (10, p. 401). It is believed, however, that the evidence presented in this paper is more definite and extensive than has previously been published.

The study of this problem is complicated by the fact that the breakdown, either of the protein-lipid complex, or of the lipids themselves, involves two changes; first the change in the original material due to the breakdown; and second, the change resulting from the accumulation of breakdown products. Kozmin (11, 12) concluded that the latter was the important factor, since the addition of unsaturated fatty acids, which are certainly among the breakdown products, caused characteristic deterioration of the gluten, and their removal restored the original quality. Sullivan *et al.* (18) disagreed with this, and held that the breakdown products must be removed and the "flour-fat" replaced. From the present study, the writers are of the opinion that even this explanation is too simple, and that the effects of deterioration can be overcome only if the protein-lipid complex can be restored to its original form. It is not believed that this can be accomplished as simply as Sullivan's results indicate, if deterioration has proceeded as far as in the samples used in the present study.

The proof of this belief depends upon the possibility of restoring the quality of a flour from which the important lipids have been removed, or in which they have been altered to the point at which the characteristic properties of the gluten are no longer apparent. The alteration or removal may be accomplished either by natural deterioration or by extraction. In the first case it must be demonstrated that the lipids have been altered or removed. The restoration of quality after the naturally occurring lipids have been removed from effective participation is likely to prove very difficult, even if the theory were correct.

Alcohol is the only solvent with which a condition resembling deterioration has been brought about; but since mere alcohol wetting causes the condition to arise, it would appear that it is not the removal of the lipids, but the alcohol treatment, that is important. Alcohol wetting may have the same essential effect as alcohol extraction, however, since it is probably the breaking of an adsorption complex, rather than the removal of the lipids from the flour, which causes deterioration. In the breaking of the complex, the lipids involved may be altered to such an extent that reunion cannot take place after the alcohol has been removed. This would explain why the more insoluble lipids added as germ tend to restore quality, but the same lipids added as extracts do not. If alcohol wetting of flour alters the flour lipids, alcohol extraction of the germ would alter the comparable germ lipids. Some method must be devised by which unaltered lipoidal compounds can be added to extracted or naturally deteriorated flour. The use of carefully prepared individual compounds offers perhaps the best method of attack, but whether any of the compounds available are those occurring in the gluten complex is an open question.

The question as to whether alcohol extraction and alcohol denaturation are essentially the same in effect has already been raised. If the characteristic properties of natural gluten can be restored by the addition of lipoidal compounds to alcohol extracted flour, the denaturation must be due to lipid removal. Denaturation has been studied chiefly by solubility decrease, and if solubility were restored, denaturation must be considered counteracted. It is quite conceivable that the same consideration might be extended to other forms of denaturation, such as that caused by heat (5). Geddes (8) has shown that heat treatment of germ has a definite effect on the lipoids of germ. It is not suggested that gluten protein denaturation is necessarily altogether a result of changes in the protein-lipoid complex, but it is believed that the possible importance of this factor should not be overlooked. In any case, the consideration of gluten as a protein substance, and the lipoids as impurities, is no longer justifiable.

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LABORATORY MALTING. I. EQUIPMENT¹

By J. ANSEL ANDERSON²

Abstract

A laboratory malting plant is described which consists of duplicate steep tanks, germination chambers and kilns, each having a capacity of eight 350-gm. samples. The two steep tanks are equipped independently with immersion heaters and thermoregulators and are cooled by a continuous flow of water from a thermostatically controlled supply tank. The two germination chambers are controlled by independent air-conditioning units. The kilns are electrically heated, the controls being adapted to give either stepwise or continuously rising temperatures. The malt in both germinators and kilns is rotated continuously in cylindrical cages. Duplicate sets of equipment were built in order to make possible simultaneous study of different malting procedures. The equipment is discussed in the light of one year's experience with it and various improvements are suggested.

Introduction

Laboratory equipment for malting a number of small samples of barley under uniform conditions was described by Harrison and Rowland (4) and to these authors must go the credit for conceiving the idea of carrying out large numbers of routine malting tests in the laboratory. Their equipment was installed at the University of Manitoba in 1927 but publication of a description of it was delayed until 1932. About two years later three malting laboratories were established in the United States, at the Universities of Wisconsin (3) and Minnesota (5) and in the Department of Agriculture at Washington (2). The laboratory malting test is thus gaining a firm foothold on this continent and if natural development takes place it may well find as wide a use in the study of barley as the baking test has found in the study of wheat.

The equipment at the University of Manitoba has been used each year for testing samples of barley produced by Canadian investigators. As the work was expanded, and with the increasing use of statistical methods for the analyses of the data obtained, it became apparent that the test was not sufficiently precise for many purposes. At the request of the National Barley Committee, investigations designed to develop equipment and methods for a more precise routine malting test were therefore undertaken in the National Research Laboratories.

Experience obtained at the University of Manitoba has already shown that the sample for the routine malting test should be kept as small as possible. In the past the test has been used in Canada for two main purposes: (a) comparison of the malting qualities of varieties; (b) testing samples produced in investigations of environmental effects on malting quality, including the effects of cultural practices and fertilizers, and studies designed to outline

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more closely the most promising malting barley areas in Canada. For both purposes a test which can be made in duplicate with a sample of less than 1000 gm. is advantageous since these samples can be produced on the relatively small plots which are used to such advantage in modern field investigations. Small samples also make it possible to use smaller malting equipment in which the problem of space variations in conditions can be more easily solved and in which it should therefore be possible to make more precise malting tests.

It is probable, on the other hand, that small samples create difficulties in malting. It seems safe to assume that the larger the sample used in the laboratory malting equipment, the more closely can the conditions of the commercial plant be reproduced, and the more closely will the resulting malt resemble commercial malt. On the other hand, it is probably enough that the malting test should estimate the quality of a sample as compared to that of a standard sample malted under the same conditions. Indeed it is doubtful whether any other sort of malting test can be used in routine work. The maltster attempts to malt each lot of barley to the best advantage and varies the malting conditions to meet the requirements of the barley. Such a variation of conditions is not, in general, permissible in scientific investigations, since if it is used it then becomes impossible to say whether the test measures the malting quality of the samples or the ability of the investigator to malt them. A variation of conditions to suit the sample is only permissible when a standard condition for all samples can be set beforehand, and when the malting process required to bring the samples to the required condition can be determined by an objective method. Steeping affords a good illustration of this point. It is not necessary in the routine test to steep all barley for the same length of time. It can be decided beforehand that all samples should have the same moisture content at the end of the steeping period, say 44%, and pilot steeping tests can be used as an objective method of determining the length of time required to bring each sample to the required moisture content. It seems doubtful, however, whether similar methods can be developed for deciding in what ways germination and kilning procedures can be modified to suit individual samples.

Under these conditions it is apparent that the laboratory test need not ape commercial practice. The test must be comparative rather than absolute, and the problem of developing it is mainly that of devising equipment for steeping, germinating and kilning under closely controlled conditions. It still remains to be shown that the results of tests of this sort are of practical value; in short, that they provide a true measure of the inherent malting qualities of the barley.

Since the known advantages of using small samples seemed to outweigh the possible advantages of using larger ones, the new equipment for the National Research Laboratories was built to accommodate 350-gm. samples, a size which provides ample malt for all useful determinations which can be made conveniently on a routine scale. To facilitate changes suggested by

experience, the units were built in such a way that parts could be readily modified without the necessity of rebuilding the whole unit. In the attempt to provide for all contingencies which could be foreseen, the equipment was made more complex than may be necessary, and certain parts of it have not yet been used to any considerable extent.

Before designing the equipment, visits were made to the malting laboratories at the Universities of Manitoba, Minnesota and Wisconsin, in order to study the existing laboratory plants. Plans for new equipment were also discussed with practical maltsters. The information and advice obtained in this way were of inestimable value, and the new equipment which was subsequently designed incorporates those features of other laboratory plants which seemed to be most useful.

The new plant has now been in operation for about a year, and although further development of equipment and method is anticipated, it seems wise to publish a description of it in order that subsequent papers on investigations made with it may be intelligible. Moreover, since the laboratory malting test is still in its infancy, and since it is to be expected that new plants will be built in other laboratories, it seems advisable to make available, for the use of others, information on laboratory malting equipment which differs in many respects from that already described in the literature.

Equipment

The malting equipment consists of duplicate steep tanks, germination chambers and kilns. Each unit has a capacity of eight 350-gm. samples and is provided with independent controls. Duplicate sets of equipment were built in order to make possible simultaneous study of different malting procedures.

Steep Tanks

Drawings of the two steep tanks and their common water-supply tank are shown in Fig. 1, and part of the equipment appears on the left-hand side of Fig. 3. A diagram of the control system is shown on the left-hand side of Fig. 2.

The steep tanks are cooled by a continuous flow of water from the supply tank which is maintained at a temperature lower than that required for steeping. Independent control for each steep tank is obtained by heating back with an immersion heater *J* (Figs. 1 and 2) controlled by a bimetallic thermoregulator *K*.

The supply tank is cooled by an evaporator coil *A* connected to a $\frac{1}{3}$ H.P. household type compressor *E*, which also provides the refrigeration for the germination chambers. The temperature of the water is controlled by a non-indicating commercial type electrical controller with a liquid filled bulb *B*. When the temperature rises a mercury switch *C* (Fig. 2) in the controller closes a circuit which opens a solenoid valve *D* on the suction line from the evaporator *A* and the automatic pressure switch, with which a household

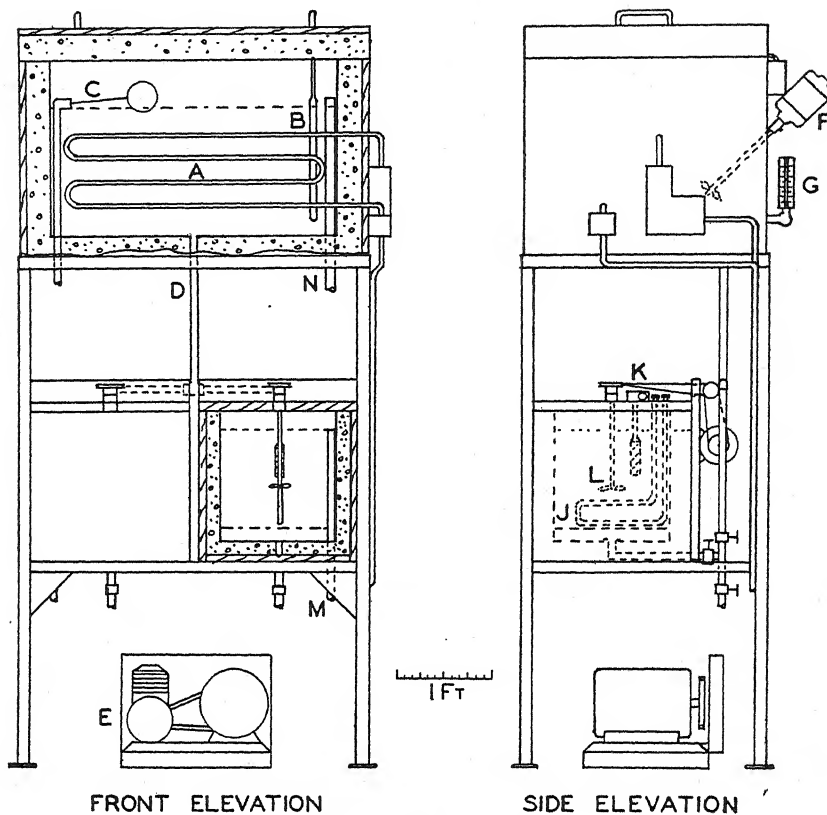


FIG. 1. Drawing of steep tanks.

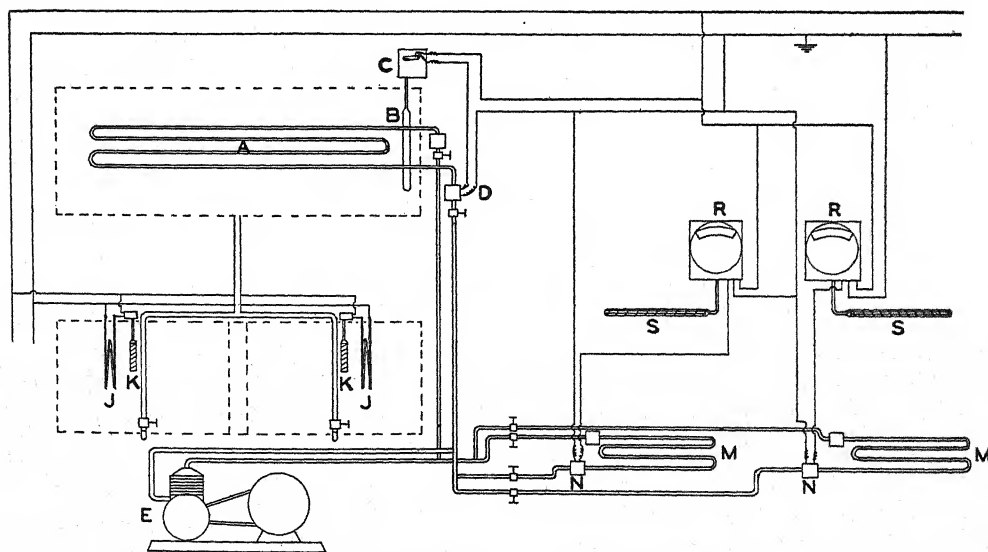


FIG. 2. Diagram of control systems for steep tanks and germination chambers.

compressor is normally provided, starts the compressor motor. The compressor operates until the temperature falls sufficiently to complete the cycle by opening the mercury switch, thus closing the solenoid valve and stopping the compressor. The temperature of the steep tank fluctuates $\pm 0.5^{\circ}\text{F}$. about a mean value which can be set to within $\pm 0.3^{\circ}\text{F}$.

All three tanks are made of galvanized iron and are insulated with cork, applied with molten asphalt, and enclosed in birch boxes. In addition to the equipment already mentioned, the supply tank is provided with a water inlet and float valve *C* (Fig. 1), a supply line for the steep tanks *D*, an overflow pipe *N*, a stirrer *F*, and an armored thermometer *G*. Each steep tank is also provided with a stirrer *L* and a removable overflow pipe and drain *M*.

Germination Chambers

A photograph of the twin germination chambers and their individual air-conditioning units is shown in Fig. 3, a drawing of a cross-section of one chamber and unit appears in Fig. 4, and a diagram of the control system is shown on the right-hand side of Fig. 2.

Both chambers and air-conditioning units are built with a galvanized iron inner case surrounded by 3 in. cork, applied with molten asphalt, and enclosed in an outer case of galvanized iron. The inside walls are painted with asphaltum paint. The ducts are also made of galvanized iron and are insulated with 1 in. of wool felt.

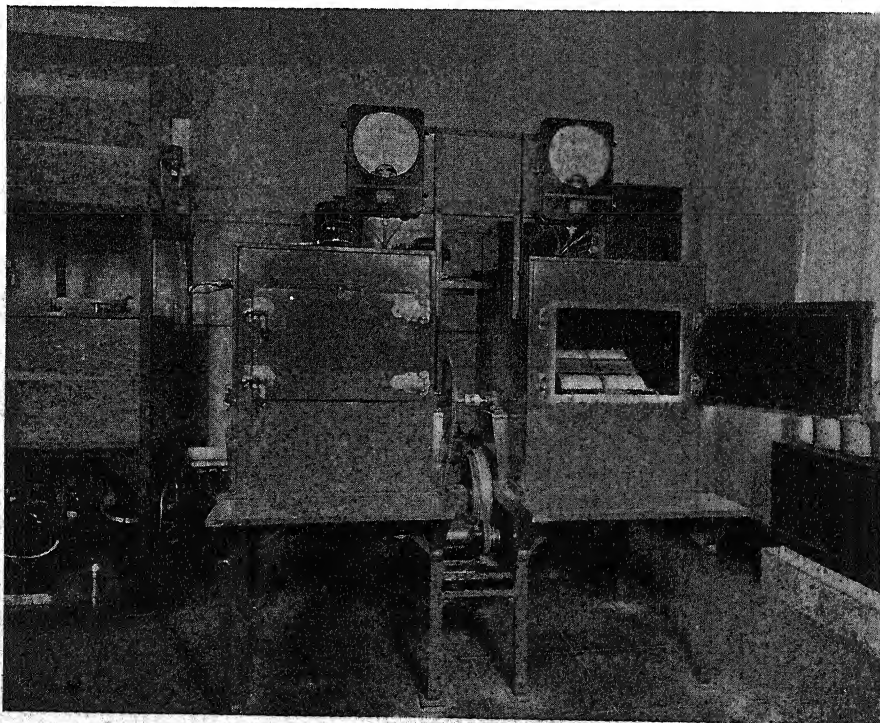


FIG. 3. *Germination chambers.*

Each germination chamber is provided with a revolving framework *U* (Fig. 4) which holds eight cylindrical malt cages, 6 in. in diameter and 6 in. long. The shafts for the two chambers are joined by a flexible coupling and are driven by a chain and sprocket drive, reducing gear and $\frac{1}{8}$ H.P. electric motor, *V* (see also Fig. 3). Three-step pulleys on the reducing gear and motor provide for speeds of one revolution in 20, 40 and 60 min. A baffle plate *T*, in the shape of a low pyramid, made of 14-mesh bronze wire and with a 2 in. metal top, is supported just below the air-supply duct in the top of the chamber and serves to distribute the air flow. The temperature control bulb *S* is installed horizontally across the chamber just above the baffle plate.

The chambers were built for revolving cages but they can also be used for stationary cages since the revolving parts are removable and brackets have been provided for a shelf. No experiments have yet been made with stationary cages.

A centrifugal fan *A* having a capacity of about 30 c.f.m. circulates air continuously through the chamber and its air-conditioning unit. The air is drawn out of the bottom of the chamber, passes into a spray chamber *G* in the bottom of the unit, in which it is cooled and humidified, and then through eliminator plates *F* which remove entrained water. It is then returned to the top of the chamber. An air-inlet *H* in the lower duct allows a small amount of fresh air to be bled into the system continuously. The spray is

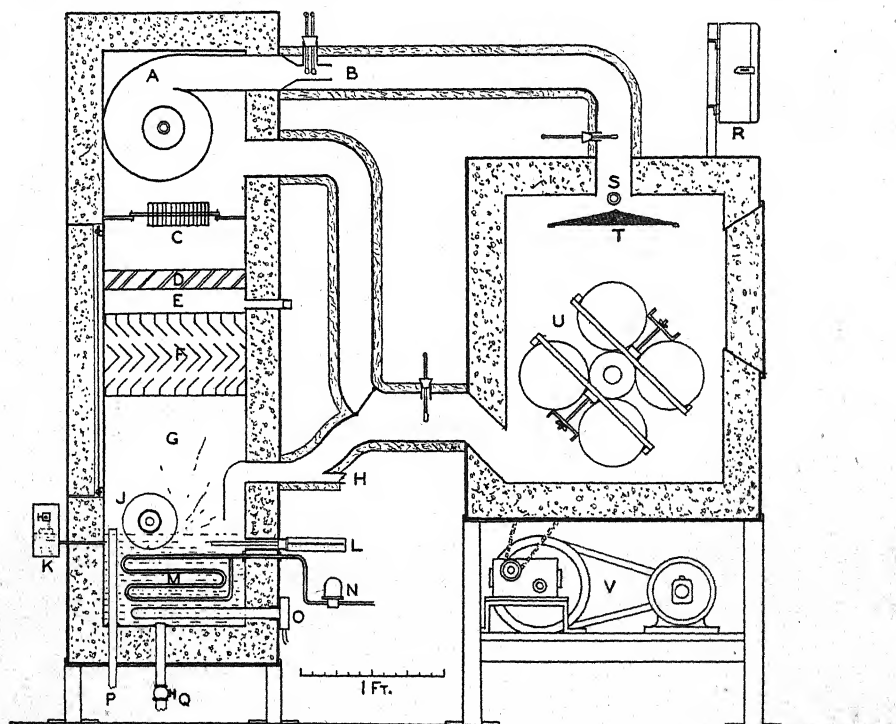


FIG. 4. Cross section through air-conditioning unit and germination chamber.

created by four rapidly revolving discs *J* which are partially immersed in a water tank. The spray rotor shafts of the two air-conditioning units are connected by a flexible coupling and are driven by one motor. The same system is used for driving the two fans.

The temperature of the chamber is controlled by a commercial type indicating electrical controller with a sensitive liquid-filled capillary bulb *S* (Figs. 2 and 4). The control system is identical with that of the water-supply tank. When the temperature rises a mercury switch in the controller *R* closes a circuit which opens a solenoid valve *N* on the suction line from an evaporator coil *M* immersed in the spray tank at the bottom of the air-conditioning unit. The compressor starts automatically and the temperature of the spray and of the air passing through it drops until the cycle is completed by activation of the controller. At ordinary room temperatures, the cycle is completed in 12 min., during which time the compressor runs for about 2 min.

When the room temperature remains reasonably constant, the temperature of the air entering the germination chamber fluctuates $\pm 0.5^{\circ}$ F. and that of the air leaving the chamber fluctuates $\pm 0.2^{\circ}$ F. The control instrument has an open scale and a setting tolerance of about $\pm 0.1^{\circ}$ F. The mean temperature of the chamber can be maintained within $\pm 0.15^{\circ}$ F. of the set temperature.

The upper duct is constricted for a short distance *B* (Fig. 4) to provide an air flow of 30 linear feet per sec., thus making it possible to measure the humidity of the air with wet and dry bulb thermometers. In the better of the two units, the relative humidity of the air leaving the conditioning unit is about 95%. As there is a rise of 1.5° to 3° F., depending on the room temperature, between the point at which the humidity is measured and the chamber outlet, the mean relative humidity of the chamber is less than 90%. In the second chamber the relative humidity is about 3% lower, presumably because of an air leak which it has been impossible to find.

In addition to the equipment already mentioned, the conditioner is provided with a water supply line controlled by an external float valve *K*, an overflow pipe *P*, a drain *Q*, an armored thermometer *L*, an immersion heater *O*, reheating units *C*, a section *E* for the bulb of a control instrument, baffles *D* to protect the bulb from heat radiations, and a by-pass.

Certain parts of the air-conditioning units are not being used at the present time, but it seems wise to show them in the drawings since they may be used in later investigations. The immersion heater *O* at the bottom of the unit was provided for obtaining more precise control of temperature by alternate heating and cooling of the spray water, but sufficiently precise control has been obtained without the use of this system. The by-pass was installed for the same reason but it has been found that higher humidities and precise control of the temperature can be obtained when all the air is passed through the spray. The heating units *C* at the top of the conditioner were installed

to provide a method of obtaining lower humidities such as might be required for withering the green malt at the end of the germination period. Experiments with this procedure have not yet been undertaken.

Kilns

A photograph of the twin kilns is shown in Fig. 5, and a drawing of a cross section through one of them is shown in Fig. 6.

A kiln consists of a galvanized iron inner case and partitions, surrounded by $\frac{1}{2}$ in. asbestos board and 1 in. Ten-Test, the whole being supported by an angle-iron framework. The inside of the kiln is divided into three parts, a heating chamber, an air-distribution chamber and a drying chamber. The heating chamber consists of a 9×9 in. chimney, located centrally at the back of the kiln, containing four 100-watt fin-strip heating elements *A* (Fig. 6). A double opening centrifugal fan *B* below the heating elements, draws air past them and blows it into the air-distribution chamber *C* from which it escapes through eight one-inch pipes *D* which direct it onto the malt. Inside the drying chamber there is a revolving framework *E* which holds eight samples

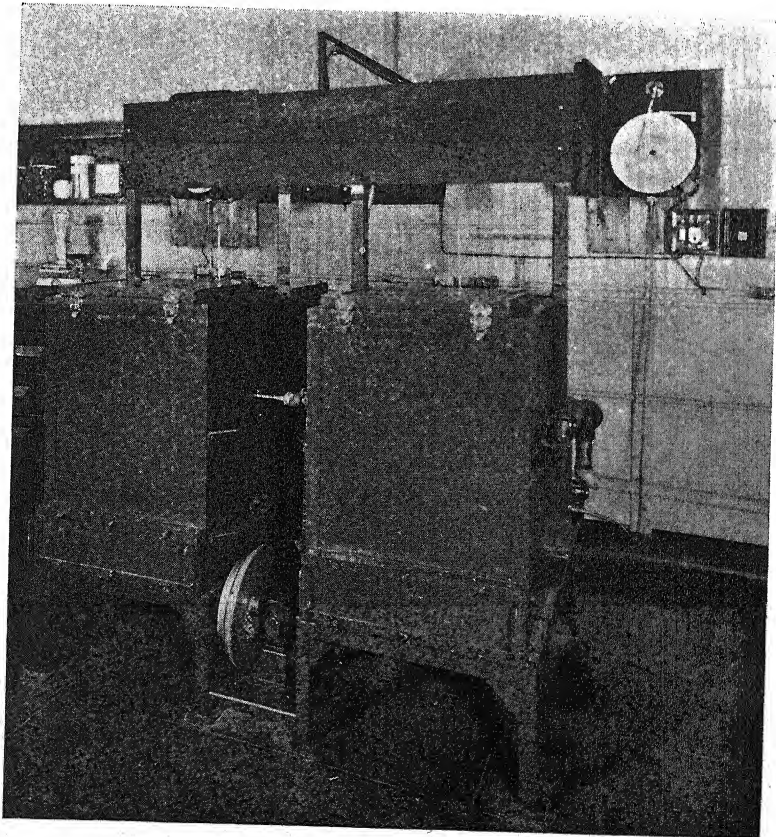


FIG. 5. *Kilns.*

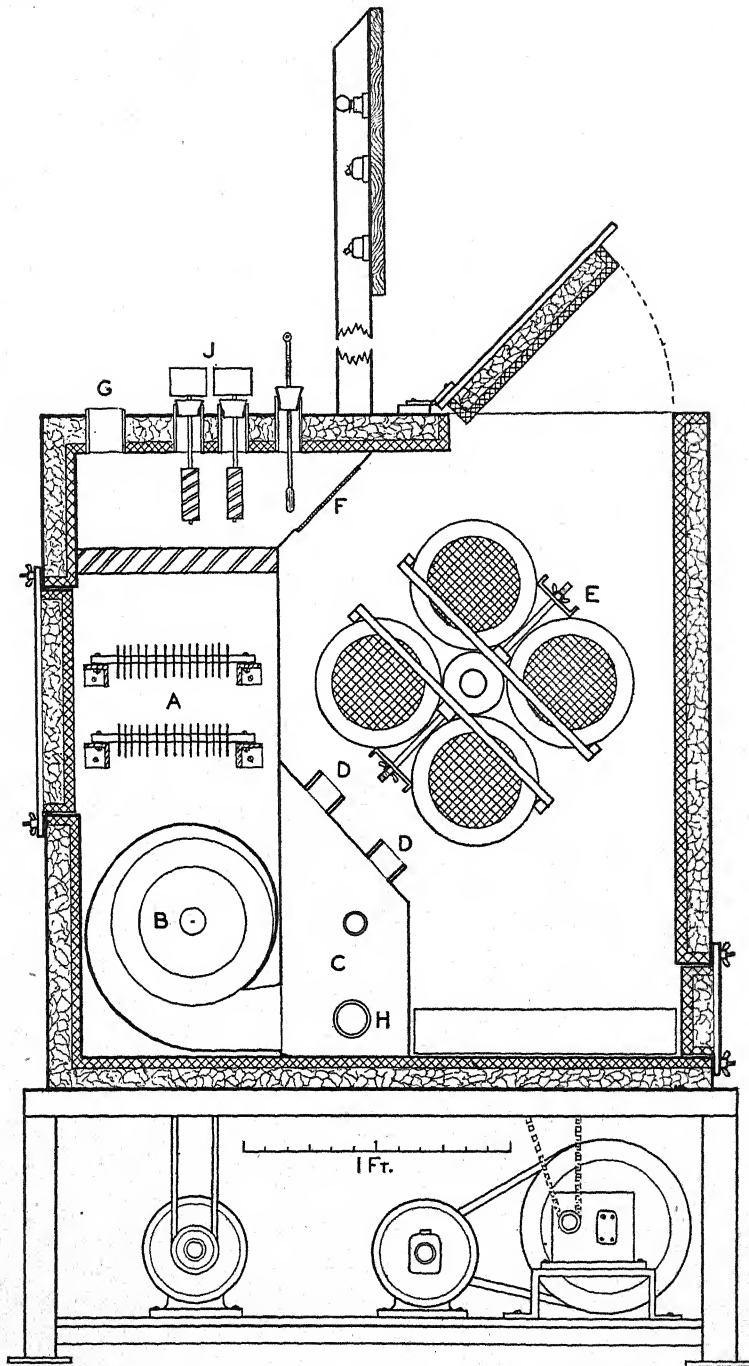


FIG. 6. Cross section through one kiln.

of malt contained in cylindrical wire-mesh cages. The air passes back from the top of the drying chamber to the top of the heating chamber, through a wire-mesh screen *F*.

A hinged lid is provided in the top of the drying chamber for inserting and removing the malt cages. A removable panel in the back of the kiln gives access to the heating elements and a similar panel is provided in the front of the kiln for withdrawing the tray from the bottom of the drying chamber.

The fan has a capacity of about 30 c.f.m. The fan shafts for the two kilns are joined by a flexible coupling and are driven by the same motor. A similar arrangement is used for driving the rotating frames. A chain and sprocket drive and a reducing gear with a three-step pulley provide for rotation rates of one revolution in 5, 2.5 or 1.25 min.

A circular duct *G*, $1\frac{1}{2}$ in. in diameter, in the centre of the top of the heating chamber allows fresh air to enter the kiln. About one-fifth of the air is bled out of the kiln for each revolution of the air by means of a $1\frac{1}{2}$ in. pipe *H* located in the side of the air-distribution box.

Two methods of temperature control have been used. The first involves the use of four bimetallic thermoregulators *J* placed in the return air duct at the top of the kiln, giving a stepwise control in four stages. The second method gives any desired time-temperature schedule. It consists of a 12-in. disc type recording thermometer, with a 48-hr. clock, modified according to the directions of Binington and Geddes (1) to form a time-temperature controller (see top right-hand corner, Fig. 5). A diagram of the control system is shown in Fig. 7. The pen arm *A* (Fig. 7), which has been cut and insulated, operates through a relay *B* and turns the heating element *C* on and off by running on and off a copper surface applied to a celluloid disc *D*. The outer edge of the copper follows the time-temperature schedule for the kiln. As the temperature rises, additional heating elements can be turned on automatically by means of reverse-acting bimetallic thermoregulators *J*. The control bulb is inserted into the air-distribution chamber of the kiln through a pipe and coupling *K*.

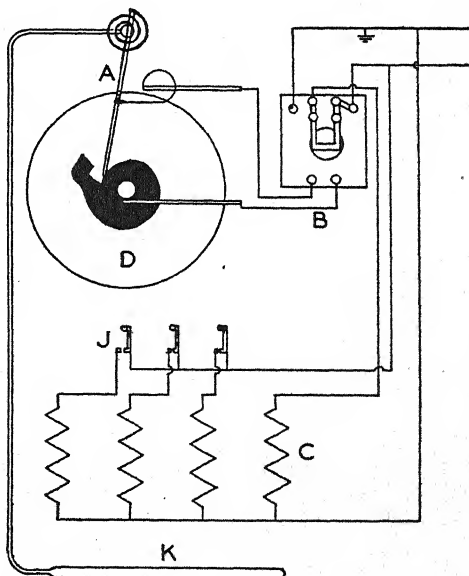


FIG. 7. Diagram of control system for one kiln.

Malting Method

The steep tanks were designed to hold eight wire-mesh cages, 6 in. in diameter and 6 in. tall. Experiments soon showed that steeping in this type of cage in running water was not satisfactory. The amount of oxygen supplied by the water was excessive, with the result that the barley "chipped" in the steep, and vigorous growth started too rapidly. It is now the practice to steep the barley in closed quart sealers. The samples are aerated once a day by draining off the water and allowing the sealers to float in the tank for one hour before refilling them with fresh water.

For the first eight hours in the germination chamber the samples are held in cylindrical wire-mesh cages in order to allow the adhering water to evaporate. The samples are then transferred to cylindrical galvanized iron cans with eight $\frac{1}{8}$ in. holes, four in the lid and four in the bottom. The samples are watered 60 hr. after being put into the germination chamber. At 120 hr. the samples are put back in the wire-mesh cages to permit some withering to take place. The germination process is terminated at 144 hr.

During the whole of the germination period the samples are rotated continuously at a rate of one revolution in 40 min. The temperature of the chamber is maintained about 5° F. below the highest temperature the malt is required to reach. Owing to the heat generated during growth, the temperature of the malt rises steadily until about the end of the fifth day when it starts to fall slowly. Good growth and modification have been obtained with a chamber temperature of 54° F.

The samples in their wire-mesh cages are transferred to the kiln in which they are rotated continuously at a rate of one revolution in 1.25 min. Various time-temperature schedules have been adopted for kilning. By making small variations in the temperature at various stages of drying, kiln-dry malt, having a moisture content of about 4%, can be obtained in any required time between 34 and 52 hr.

When the kiln was designed it seemed probable that most of the roots would be rubbed off during kilning. Experience has shown, however, that the loss of roots during kilning is almost negligible and thus an estimate of the malting loss due to root growth can be obtained for each sample.

The kilned malt is polished by kneading it by hand in a small sack and the roots are subsequently removed by sifting.

Discussion

Experiments have shown that steeping the barley in running water under conditions in which the proportion of water to barley is very much larger than it is in commercial plants, is not satisfactory. For this reason the design of the present steep tanks cannot be considered good. A far larger amount of refrigeration and water is used to keep the tanks cool than would be required if they were redesigned. Those who contemplate building new equipment might well consider the advisability of building the evaporator into the steep tank, providing a float valve to keep the water level constant, and making up only that water which is periodically drained from the samples.

It seems possible that higher relative humidities might be beneficial in the germination chambers. The use of cages having only eight small holes prevents loss of moisture by evaporation almost entirely, while permitting sufficient interchange of air to keep the carbon dioxide content of the air in the cages from becoming a limiting factor for growth. It must be admitted, however, that this method of restricting evaporation has its disadvantages. The temperature of the actively growing malt rises several degrees above that of the air outside the cages. While the malt temperatures thus follow a similar course to those obtained in commercial plants, it is apparent that accurate control of temperature, humidity and carbon dioxide content of the air in the cages is not obtained. Whether precise and independent control of these factors will be required for a satisfactory malting test remains to be investigated.

The insufficiently high humidities in the chamber are a result of the design of the equipment. The main heat load is that resulting from heat leakage into the chamber from the room. This load must be taken care of by the same system that humidifies the air. Thus the temperature of the spray water is lower than that required in the chamber to an extent which is dependent upon the room temperature. Since the temperature of the spray determines the dew point of the air leaving it and since a temperature increase occurs between the spray and the chamber, the relative humidity of the air in the chamber cannot be maintained close to the saturation point. The difficulty could be overcome by using a separate cooling system to take care of the heat leakage from the room. It appears that this might best be done by making double-walled chambers and installing auxiliary cooling coils between the walls.

Higher relative humidities could also be obtained by building the air conditioner and chamber in one unit so that less temperature rise would occur between spray and chamber. Although this point was apparent when the present equipment was designed, the advantage of being able to use different types of chamber with the same air-conditioning unit seemed to outweigh the possible advantage of a gain in humidity resulting from building a single unit which would have to be discarded entirely if the germination chamber required modification.

It is felt that sufficient experience has not yet been obtained with the kilns to make adequate discussion of them possible, but some observations seem permissible. The kilning method differs entirely from that used in commercial plants, since only a small part of the air passing through the drying chamber passes through the malt. In these circumstances a recirculating system with a "bleed-in" for fresh air seems permissible. The "bleed-in" keeps down the relative humidity of the air, which is one of the factors in drying efficiency. The fact that the air that has passed the malt enters the heating chamber again must not be considered as similar to a hypothetical use of return air in commercial kilns, because the condition of this air both

with respect to humidity and temperature is not similar to that of the comparatively smaller amount of air which actually passes through the malt in a commercial kiln.

The use of continuously revolving cages lends itself to uniform conditions of drying for all samples and, owing to the thorough mixing, for all parts of each sample. Its disadvantage is that the air does not actually pass through the malt but only over continually new exposed surfaces of the sample. There is some evidence that this method makes it difficult to obtain sufficiently rapid drying during the early stages of kilning, with the result that excessive growth continues for a short time on the kiln and malting loss is increased.

The whole plant has been in operation almost continuously, day in and day out, for eight months. No samples have yet been lost through mechanical failure in the equipment. On the whole, it appears that the plant is satisfactory for the purpose for which it was designed, namely, the development of equipment and methods for a routine laboratory malting test.

Acknowledgments

The writer has borrowed freely from the designs of laboratory malting equipment already in existence and takes this opportunity of acknowledging his debt to the staffs of the malting laboratories at the Universities of Manitoba, Minnesota and Wisconsin. The author is also indebted to Mr. P. J. Dax of the Canada Malting Company Limited, Montreal, and to Mr. D. S. Kaufman of the Dominion Malting Company Limited, Winnipeg, for many useful criticisms of the first designs for the equipment.

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GENETICAL STUDIES ON MUTANTS IN THE PROGENY OF HEAT-TREATED BARLEY¹

By F. H. PETO²

Abstract

Heat treatments were applied to barley seeds and 10 different mutant characters were observed in the progeny, *viz.*; xantha₁ and 2, dwarf_{1,2,3} and 4, virescent₁ and 2, chlorina and albino. Typical Mendelian ratios were not obtained in the first segregating generation owing to the small size of the sector affected in the generation of treatment. In the second and third segregating generations, good fits were obtained in all cases to either monohybrid or dihybrid ratios. Both 3 : 1 and 15 : 1 ratios were observed in lines segregating for xantha₁ and albino characters. The postulation of the duplicate factor hypothesis was necessary to explain this situation. Chlorina and dwarf mutants segregated in all the cases investigated as simple Mendelian recessives. One virescent strain was believed to have arisen through plastid mutation and was maternally inherited.

The heat treatment significantly increased the natural mutation rate for the xantha characters but apparently had no effect on the albino mutation rate. Dwarf, virescent and chlorina mutants were observed in the segregating generation after heat treatment, but were not detected in untreated populations.

Introduction

Previously reported experiments (7, 8, 9) consisting of heat treatments of dormant and germinating barley seed have demonstrated that many chromosomal mutations were induced in the root tips of the young plants. These chromosomal mutations included fragmentation, translocation, interchange, deficiency, duplication, as well as doubling of the whole chromosome complement. While it was shown that many of these abnormal nuclei were rapidly eliminated on further plant development, it was thought that certain of these chromosomal mutations occurring in the primordia of the spikelets might survive through the gametophytic generation and influence character expression in the next sporophytic generation. It was also recognized that numerous recessive gene mutations, which would not be detected until the segregating generation, could have been induced by the heat treatment. The present paper therefore deals mainly with the evidences of Mendelian segregation in the progeny of heat-treated plants. Cytological studies are also under way to distinguish as far as possible between the plant mutants which were the result of structural chromosomal changes (duplication, deficiency, translocation, etc.), and those which were the result of gene mutations. These studies have not yet been completed.

Although few data have been published on the influence of temperature on the gene mutation rate, several investigators have dealt with the influence of temperature on the chromosomal mutation rate. Randolph (10) applied heat treatments in the early zygotic divisions but found no evidence that the high temperatures were effective in causing gene mutations, although he

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obtained doubling of the chromosome sets and chromosomal deficiencies and translocations. Navashin and Shkvarnikov (5) heat treated seed of *Crepis tectorum* at 54–55° C. for 20, 40 and 44 days and obtained a large number of chromosomal translocations in the root tips of the plants which survived. The same authors in a more recent contribution (13) on a similar experiment conclude that temperature and moisture are the principal climatic factors which influence the chromosomal mutation rate. They also reported a segregation of 49 albinos in 369 plants in the progeny of one of their heat-treated plants. Kirnossowa (4) studied the influence of high temperature under controlled humidity conditions on the chromosomal mutation rate in *Crepis tectorum* and concluded that the habits and behavior of the seedlings as well as the character of chromosome mutations were similar to those occurring after long storage of seed under normal conditions.

Methods

Glass capsules were filled with approximately 300 seeds of first generation registered O.A.C. 21 barley of Wiener's strain, sealed to prevent escape of moisture, and put in an oven at 85° C. The treated seeds were sown in flats, grown for three weeks in the greenhouse, and then transplanted to the field during the last week of April, 1935. These plants were carefully observed during growth in order that the occurrence of any abnormalities might be recorded. At harvest time any plants that exhibited partial sterility or were abnormal in any respect were separated from the remainder to determine whether their progeny exhibited a higher frequency of mutation.

Segregation for heat-induced mutations occurred in the immediate progeny of the plants grown from the heat-treated seeds. The symbol D_1 has been used to designate this generation, and subsequent generations were designated D_2 , D_3 , etc. It should be noted that segregation occurs in the D_1 generation in contrast to its occurrence in the F_2 following hybridization.

The D_1 seed was sown thickly in greenhouse benches and was examined two weeks after seeding when the plants were about five inches high. At this stage all the types of mutants could be detected. Any lines that did not possess mutant plants were immediately discarded and another lot of plants sown. In this manner it was possible to examine critically more than a quarter of a million plants during the winter, utilizing only 140 square feet of bench space.

The χ^2 test for goodness of fit was applied to the totals of all the segregating lines of each family in a given generation. In order to avoid the necessity of recording the ratios and goodness of fit for each line, the χ^2 test of homogeneity was calculated. The fits were considered good in all instances where the χ^2 value was less than the 5% point.

Immediate Effects of Heat Treatments on Germination and Plant Development

In the first experiment, the effects of heat treatments at 85° C. for 90, 100, 105 and 110 minutes on the rate and the percentage germination were as shown in Table I and Fig. 3. Germinability and seedling vigor were

TABLE I
PERCENTAGE GERMINATION AFTER HEAT TREATMENT AT 85° C. FOR DIFFERENT DURATIONS OF TREATMENT

Days after seeding	1	2	3	4	5	6	7	8	9	10
Untreated	0	95	95	95	95	95	95	95	95	95
90 min. treatment	0	0	22	46	58	69	74	74	76	80
100 min. treatment	0	0	0	11	28	40	47	47	48	48
105 min. treatment	0	0	0	0	5	17	18	18	19	21
110 min. treatment	0	0	0	0	0	0	0	0	0	0

gradually reduced with increasing duration of treatment. On the basis of these results it was decided to adjust the period of heating to reduce the germinability to 50%, but it was found to be very difficult to obtain consistent values in spite of careful control of temperature. One lot of heated seed (Lot 1) was finally obtained which germinated 46% after 105 minutes' treatment at 85° C. and 1451 plants from this lot were used in this experiment. In addition 556 plants from Lot 2 were used, but the germinability in this lot was 82% in spite of being subjected to 85° C. for 115 minutes. The data for treatments in which the progeny was analyzed are shown in Table II.

TABLE II
IMMEDIATE EFFECTS OF HEAT TREATMENTS AND THE MUTANT LINES OBSERVED IN THE D₁ GENERATION

	Lot 1	Lot 2
Temperature of treatment	85° C.	85° C.
Duration of treatment	105 min.	115 min.
Seeds treated	3630	680
Germination	46%	82%
Number plants grown	1451	556
Number abnormal plants	72	42
Number plants with partially sterile heads	222	96
Number plants in D ₁ generation examined	227,651	5796
Number lines in D ₁ segregating for xantha ₁	3	1
Number lines in D ₁ segregating for xantha ₂	1	0
Number lines in D ₁ segregating for chlorina	1	0
Number lines in D ₁ segregating for albino	3	1
Number lines in D ₁ segregating for dwarf ₁	1	0
Number lines in D ₁ segregating for dwarf ₂	1	0
Number lines in D ₁ segregating for dwarf ₃	1	0

In spite of the wide differences in germinability between Lots 1 and 2, the percentages of abnormal seedlings were similar, 4.9% in Lot 1 and 7.6% in Lot 2. In one of the abnormal seedlings (No. 232) only the first seedling leaf was normal, the remainder were light green. This abnormality was considered to be the result of a plastid mutation, since in subsequent generations this character seemed to be maternally inherited and all the progeny were affected. Three seedlings possessed white stripes on the leaves. One of these seedlings died; the other two appeared normal on further development.

Two plants at a later stage developed leaves with broad yellow stripes as shown in Fig. 5. The remainder of the abnormal seedlings were smaller and less vigorous, and in a few the leaves were slightly darker green than normal and strongly curled.

Stadler (14) observed a yellow striping in barley in the generation of treatment with X-rays and ascribed it to a direct cytoplasmic effect of the treatment. Imai (3) observed sporadic white and yellow striping in barley and although a stimulating gene was involved, the immediate changes were believed to be due to plastid mutations. Imai also found the rate of plastid or exo-mutation was influenced by the environment. The immediate occurrence of white and yellow striping after heat treatment in this experiment is therefore probably the result of plastid mutations. However, the possibility of striping being the result of gene or chromosomal mutations should not be overlooked. Since the progeny of plants that exhibited striping did not segregate for this character, it can be assumed that the plants were homozygous normal. Consequently, to produce immediate genetic striping from heat treatments, gene mutations or chromosome aberrations must have occurred simultaneously in identical regions of homologous chromosomes. Previous studies (8) have indicated how homologous deficiencies could be induced by heat treatment. Types of somatic chromosome associations resulting from fracture and re-attachment of chromatids have been seen; on separation these would likely result in homologous deficiencies. Therefore, genetic striping could arise in such a manner.

It was concluded that the majority of abnormalities observed in the generation of treatment were the direct effect of the heat treatment on plant development, rather than the result of induced genetic mutations. The few cases of chlorophyll deficiency observed were attributed to plastid mutation.

Segregation in the D_1 Generation and Description of Mutants

Thirteen segregating lines were observed in the D_1 generation. Four of these segregated for xantha₁ character, one for xantha₂, one for chlorina, four for albino and three lines for three different types of dwarfs. An examination of the D_1 ratios in Table V shows that only a few approximate any Mendelian expectation. This irregularity in the ratios was undoubtedly the result of variations in size of the heterozygous sector in the generation of treatment. The size of the sector in turn depends on the stage of development of the embryo and the location of the original mutant cell. In the D_1 of Plants 346, 24, 14, and 375, segregation occurred only in the progeny of one out of four or five spikes harvested from each plant. In addition it can be shown that only a small sector of the spike was affected in Plant 14. This situation made it necessary to determine Mendelian ratios in the D_2 and D_3 generations.

The descriptive terms used for the chlorophyll-deficient seedling types are those described by de Haan (1) and previously used by Nilsson-Ehle (6), Hallquist (2), Robertson and Deming (12) and others. The albino seedlings

(Fig. 1) are pure white and live only until the reserve food in the endosperm is exhausted. The xantha seedlings are so deficient in chlorophyll that they live only slightly longer than the albinos. Xantha₁ seedlings are a very pale yellow while xantha₂ seedlings are greenish yellow. Analysis of leaf color at the midpoint of the blade with the Lovibond tintometer illustrates the differences in intensity of the blue and yellow components between the two xantha types (Table III).

The chlorina mutants were extremely variable in vigor and chlorophyll content. They were classified into three types. Type 1 seedlings were fairly vigorous. The leaves were slightly lighter green than normal

and many of the first seedling leaves had white tips and bases. These seedlings survived for two or three months in the greenhouse but never developed beyond the seedling stage. Type 2 seedlings were uniformly yellow in color and were only very slightly darker yellow than xantha₂. These seedlings died two to three weeks after seeding. Type 3 seedlings were very weak and small; the color of the seedling leaf graded from light green in the upper half to white at the base. These seedlings usually died somewhat earlier than those of Type 2. It should be pointed out that these three types are believed to be genetically identical, at least for the chlorina factor, since the segregating ratios were uniformly 3 : 1 in spite of the wide variations in the proportions of the three types of recessive seedlings. The most plausible explanation of this condition is that the phenotypic expression was very unstable and slight differences in nutrient supply arising from differences in plumpness of the seed would alter character expression. Actually the seed producing Type 3 appeared to be more shrunken than the seed producing either of the other two types. Typical specimens of the three types are shown in the fifth, sixth and seventh seedlings of Fig. 1.

Three D₁ lines segregated for dwarfs. The dwarf₁ plants observed in the progeny of Plant 726 were characterized by a slow growth rate, slate-green leaf color and profuse tillering. The plant shown in Fig. 6 is typical. The tillers developed until the shot-blade stage but spikes were rarely formed. Only one short compact spike was formed and it was sterile.

The dwarf₂ type was observed in the progeny of Plant 730. The dwarfs found in the D₁ and D₂ generations died in the seedling stage, but those occurring in the D₃ generation are growing very vigorously three months after seeding. The second of the seedlings represented in Fig. 2 was typical

TABLE III
COLOR ANALYSIS OF NORMAL AND CHLOROPHYLL DEFICIENT BARLEY LEAVES WITH A LOVIBOND TINTOMETER

—	Lovibond units	
	Blue	Yellow
Normal leaves	7.6	14.3
Xantha ₁ (811-31-1)	0.9	9.5
Xantha ₁ (730-1)	0.8	9.0
Xantha ₂ (22-6-8)	3.6	15.1
Chlorina (14 Type 1)	5.4	19.0
Chlorina (14 Type 2)	3.6	13.3
Chlorina (14 Type 3)	3.6	13.0

of this generation. The seedling leaves were slate-green on emergence and had an undulating surface. Later leaves resembled the normal more closely and tillering was profuse and occurred much sooner than normal. The differences between the dwarf₁ and dwarf₂ mutants were not marked and further study might reveal that they are genetically identical.

A single specimen of the dwarf₃ type (Fig. 7) was observed in the progeny of plant 842 and was strikingly different from normal barley. A comparison

TABLE IV
COMPARISON OF DWARF₃ AND NORMAL BARLEY PLANTS
AT TWO MONTHS OF AGE

—	Dwarf ₃	Normal
Height of plant	5 in.	25 in.
Number of leaves	8	6
Width of leaves	9-19 mm.	5-14 mm.
Length of leaves	59-90 mm.	110-193 mm.

of certain plant measurements between the dwarf₃ plant and a normal barley plant at two months of age is given in Table IV. Although this mutant remained very short, a very large number of tillers and leaves were formed and eventually spikes emerged from all these tillers. The plant was self sterile and

the cause was apparent when the florets were examined (Fig. 4). The anthers were shrunken and empty of pollen, and the lodicules and ovules were also under-developed. A large number of mutant florets were pollinated with normal pollen; three apparently normal seeds were produced but these failed to germinate. The chromosomes of the root tips were examined but no abnormalities were detected.

The dwarf₄ mutant was first detected in the D₃ generation from Plants 726 and 811. In the former it occurred in a line segregating for xantha₁ and in the latter in a line segregating for dwarf₂. The dwarf₄ mutants resembled the dwarf₂ mutants on first emergence because of their slate-green color and undulating leaf surface. However, the dwarf₄ mutants never developed beyond the first seedling leaf stage although they remained living for many weeks. A specimen is shown in Plant 3 of Fig. 2.

A virescent₁ strain of barley originated from Plant 232 in which a plastid mutation was believed to have occurred in the embryo of the heat-treated seed. Chlorophyll development was affected in all but the first seedling leaf as previously described. Fifty-three seeds were obtained on the only spike which developed and all the plants grown from these were virescent. The seedlings were light yellow on emergence but additional chlorophyll soon developed in the upper portions of the leaves. When the plants were grown at moderately high temperatures (about 70° F.) in the greenhouse, with winter daylight supplemented by artificial illumination at night, they gradually approached normal development but remained light green in color. A considerable amount of seed was obtained under these conditions. Under the much cooler field conditions of spring the plants developed very little chlorophyll and died before reaching maturity. Since all the plants in the D₁

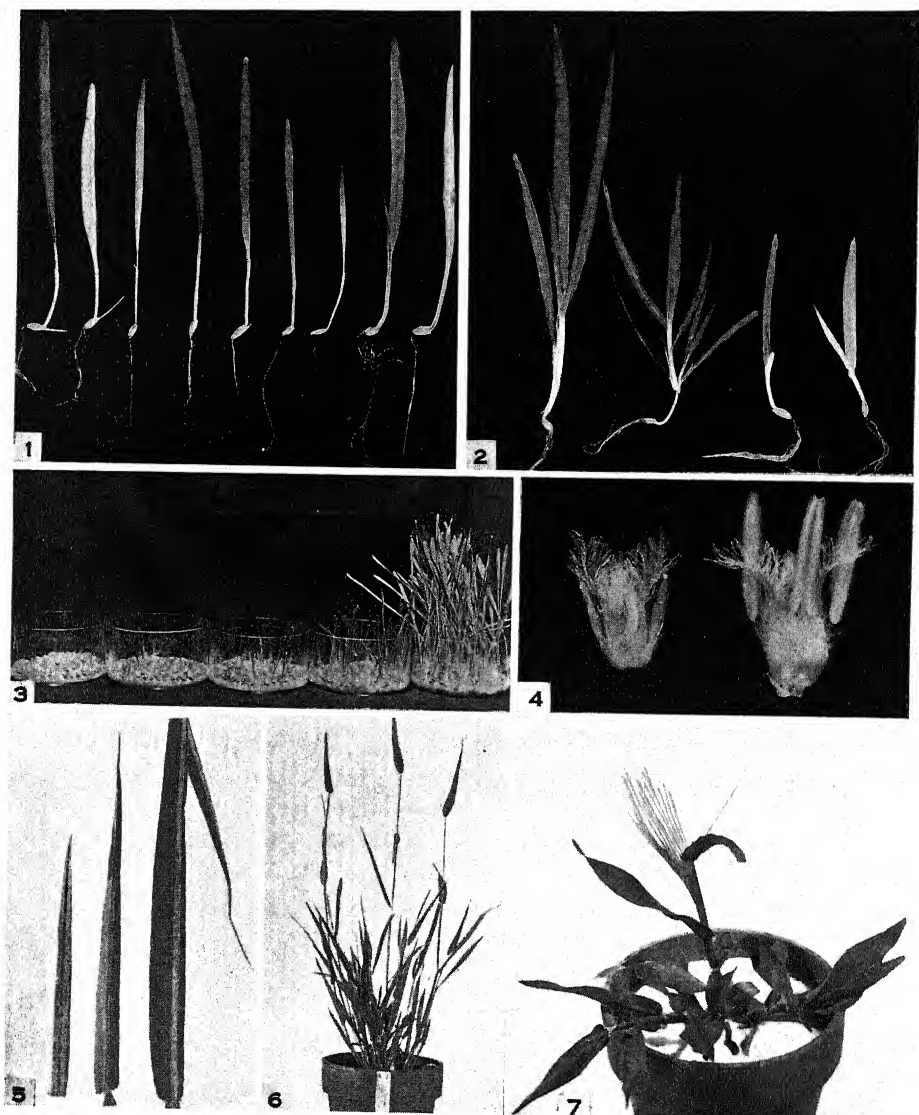


FIG. 1. Normal and chlorophyll deficient seedlings; left to right, normal (811-31), *xantha*₁ (811-31), *xantha*₂ (24-6-9), normal (14-14-16), chlorina Type 1 (14-14-16), Chlorina Type 2 (14-14-16), chlorina Type 3 (14-14-16), normal (1700-20). FIG. 2. Normal and mutant seedlings; left to right, normal (730-13-3), *dwarf*₂ (730-13-3), *dwarf*₄ (811-31-7), virescent (232-13). FIG. 3. Germination and early growth after heat treatment at 85° C. for 110, 105, 100 and 90 minutes and check. Germination percentages are 0, 21, 48, 80, and 95 respectively. FIG. 4. Left, floret from *dwarf*₃ mutant. Right, floret from normal plant, $\times 6.5$. FIG. 5. Striping in barley leaves induced in the generation of heat treatment. FIG. 6. *Dwarf*₁ mutant, $\times 1/10$. FIG. 7. *Dwarf*₃ mutant, $\times 1/5$. The numerals enclosed in brackets are the identification numbers of the segregation lines.

and D_2 generations were mutants, this is believed to be another instance of maternal inheritance of a plastid mutation. Reciprocal crosses with normal barley are being attempted this winter to test this interpretation.

Xantha₁

Segregation in the D_2 and D_3 Generations

A complicated situation was found in the D_2 and D_3 generations of lines segregating for this character. In different families of both generations, good fits as established by the χ^2 test were obtained for monohybrid (3 : 1) as well as dihybrid (15 : 1) ratios as shown in Tables V and VI. This situation may be accounted for by postulating the presence of duplicate factors for the *xantha₁* character. A plant heterozygous for both of these factors should segregate 15 normals to one *xantha₁* and in the next generation 4/15 of the lines should give the same ratio, an equal number should segregate 3 : 1, and 7/15 of the lines should breed true for normal. This test of the duplicate factor hypothesis is at present being made. Monohybrid ratios are expected only from plants which are heterozygous for one of the factors and homozygous recessive for the other. Consequently a single recessive mutation in the generation of treatment could give dihybrid or monohybrid ratios in the D_1 depending on whether the treated plant was already heterozygous or homozygous recessive for the other duplicate factor. In one instance the dihybrid ratio appeared for the first time in the D_3 of Family 811 that had previously segregated only in the monohybrid ratio. Sixteen D_2 lines had segregated 3 : 1 as did seven of the eight D_3 lines. The other line exhibited a ratio which gave a better fit to the 15 : 1 than to the 3 : 1 ratio. A reverse mutation from recessive to dominant in the D_2 , or cross pollination of genotypes which were homozygous recessive for different duplicate factors, could account for this situation. The first explanation is the more probable, since cross pollination in the greenhouse is believed to be rare.

Segregation for the dwarf₁ character was observed for the first time in the D_3 of 811 (Table VI), in material which was expected to segregate only for *xantha₁*. It is reasonably certain that the mutation did not occur in the D_2 since a single mutation in this generation could not give four segregating lines in the next generation. Consequently, it must have occurred either in the generation of treatment or the D_1 .

Xantha₂

This character segregated as a simple Mendelian recessive. Four segregating lines in the D_2 and 22 lines in the D_3 were studied. Excellent fits to the 3 : 1 ratio were obtained for the totals and the families were shown to be homogeneous.

Chlorina

In spite of the difficulties caused by the variable phenotypic expression of this character, satisfactory fits to the 3 : 1 ratio were obtained in both the D_2 and D_3 generations. The χ^2 values of the totals of five lines in the D_2 and 26 lines in the D_3 as well as the χ^2 values for the test of homogeneity were all well below the 5% point.

TABLE V
SEGREGATION RATIOS IN THE D₁ AND D₂ GENERATIONS AFTER HEAT TREATMENT

D ₁ Segregation				D ₂ Segregation									
Treated plant	Normals	Mutants	Character	No. of lines grown	Seg. lines	Total normals	Total mutants	Character	Goodness of fit			Test of homogeneity	
									Ratio	χ ²	5% point	χ ²	5% point
346	3	1	Xantha ₁	3	{ 1	65	13	Xantha ₁	3 : 1	2.89	3.84	18.27	25.00
811	208	9	Xantha ₁	208	16	22	1	Xantha ₁	15 : 1	0.14	3.84		
831	223	1	Xantha ₁	20	0	483	172	Xantha ₁	3 : 1	0.56	3.84		
1340	12	3	Xantha ₁	11	3	145	15	Xantha ₁	15 : 1	2.67	3.84	1.11	5.99
24	28	4	Xantha ₂	28	4	67	26	Xantha ₂	3 : 1	0.45	3.84	2.33	7.81
14	30	2	Chlorina	28	5	81	23	Chlorina	3 : 1	0.46	3.84	1.90	9.49
232	0	53	Virulent ₁	10	0	0	366	Virulent ₁					
375	10	2	Albino	10	{ 1	70	19	Albino	3 : 1	1.85	3.84		
926	80	1	Albino	46	1	90	2	Albino	15 : 1	2.61	3.84		
1055	396	1	Albino	93	0	66	5	Albino	15 : 1	0.08	3.84		
1700	102	3	Albino	95	3	53	13	Albino	3 : 1	0.99	3.84	2.33	5.99
726*	196	11	Dwarf ₁	196	1	16	5	Dwarf ₂	3 : 1	0.01	3.84		
730	284	1	Dwarf ₂	19	0								
842	153	1	Dwarf ₃	19									

* 196 D₂ lines examined but mutant identification uncertain, see D₃.

TABLE VI
SEGREGATION IN THE D₃ GENERATION FROM SELECTED D₂ LINES

D ₂ Segregation				D ₃ Segregation									
D ₂ line	Nor- mals	Mu- tants	Character	No. lines grown	Seg. lines	Total normals	Total mutants	Character	Goodness of fit			Test of homogeneity	
									Ratio	χ ²	5% point	χ ²	5% point
B11-31	28	9	Xantha ₁	25	{ 10 4 7 1 }	468 137 436 102	159 49 137 6	Xantha ₁ Dwarf ₁ Xantha ₁ Xantha ₁	3 : 1 3 : 1 3 : 1 15 : 1	0.04 0.18 0.36 0.09	3.84 3.84 3.84 3.84	9.73 0.19 1.91	16.92 7.85 12.59
B11-54	9	6	Xantha ₁	9	{ 20 10 12 13 6 1 }	1049 487 637 704 558 487	359 147 239 259 171 146	Xantha ₁ Xantha ₂ Xantha ₂ Xantha ₂ Chlorina Chlorina	3 : 1 3 : 1 3 : 1 3 : 1 3 : 1 3 : 1	0.19 1.11 2.43 1.85 0.92 0.96	3.84 3.84 3.84 3.84 3.84 3.84	23.38 8.46 5.80 8.98 8.34 4.07	30.14 16.92 19.68 21.03 21.03 11.07
24-6	14	9	Xantha ₁	29	{ 7 3 1 1 }	201 308 83 68	1 104 24 19	Viresent ₂ Dwarf ₁ Dwarf ₁ Dwarf ₂	3 : 1 3 : 1 3 : 1 3 : 1	0.01 0.38 0.46 0.76	3.84 3.84 3.84 3.84	4.71 0.96	12.59 5.99
24-8	18	5	Xantha ₂	18	{ 1 1 1 }	23	5	Xantha ₁	3 : 1				
14-14	18	6	Xantha ₂	18									
14-14	18	4	Chlorina	18									
14-23	18	4	Chlorina	18									
375-9*	70	19	Albino	10									
726-85		?	(Dwarf ₁ in D ₁)	18									
730-13	16	5	Dwarf ₂	9									

* One of the six D₃ lines in the progeny of the 375-9 possessed 8 xantha₁ plants in addition to 78 normals and 16 albinos.

Albino

Satisfactory fits to both 3 : 1 and 15 : 1 ratios were obtained for the albino character. Two lines segregated for each ratio in the D_2 generation. The same duplicate factor hypothesis advanced to explain the ratios found in $xantha_1$ should be equally valid for this material. In the D_3 of plant 375 one of the six lines segregating 3 : 1 for albino possessed 8 $xantha_1$ plants in addition to 78 normals and 16 albinos. Presumably the $xantha_1$ plants arose from a mutation in the D_2 since this conspicuous character could not have been overlooked in the D_1 . In addition, one virescent seedling was found in another line with 201 normal plants. This mutant appears to be very similar to the maternally inherited virescent₁ character found in the progeny of Plant 232, but its identity requires further checking.

Dwarfs

Positive identification of the different dwarfs in the early seedling stage was difficult until all the different types had been observed and carefully studied. This accounts for the failure to obtain reliable ratios in the D_2 of Plant 196. However, enough experience had been acquired by the time the D_3 generation was examined to make positive identification possible, and seven lines with the dwarf₁ character and three lines with the dwarf₄ character were found. All of these lines gave satisfactory fits to the 3 : 1 ratio. While it is certain that the dwarf₁ mutant occurred in the generation of treatment, the time of occurrence of dwarf₄ mutation is in doubt.

Only one segregating line of the dwarf₂ mutant in the D_2 generation of Plant 730 was found. The observed ratio gave a very good fit to the 3 : 1 ratio but the population was too small for the results to be conclusive. However, a larger population in the next generation gave a very good fit to the same ratio. $Xantha_1$ plants were observed in another line of the same family and it is believed that the mutation occurred spontaneously in the previous generation.

It has been impossible to determine the mode of inheritance of the dwarf₃ mutant. Nineteen D_2 lines were grown but none segregated for this character.

Frequency of Mutation in Heat-treated and Unheated Barley

Analysis of the D_1 generation of Lot 1 after heat treatment indicated that sectorial mutations of various kinds had occurred in 11 out of 1451 plants as shown in Table II. $Xantha_1$ and albino mutants were the most frequent, three of each being observed. The size of the sectors involved must have been very small as shown by the excess of normals in the D_1 ratios and by the fact that never more than one head per plant segregated. This would account for the relatively low frequency of mutant plants in the total D_1 population from Lot 1 in which the frequency of $xantha_1$ was 1 in 17,500, and of albino was 1 in 45,500. Since it was impossible to determine the frequency of occurrence of mutant sectors in a natural population, the only basis for comparison was between the frequencies observed in the D_1 after heat treatment and the frequency in the same strain grown under natural conditions.

More than $1\frac{1}{2}$ million untreated plants were examined in the field to determine the number of mutants in a natural population. The mutants in this material were derived from the accumulation of heterozygous plants from mutations in many previous generations. It would assist greatly in the interpretation of the results of this experiment if it were possible to calculate accurately the proportion of mutants derived from mutations in the previous generation and make the necessary correction for the proportion of the mutants derived from the accumulation of heterozygotes from earlier generations. This was not possible for two reasons: First, on account of the unknown variation in the mutation rate from year to year; second, on account of the occurrence of both monohybrid and dihybrid segregation ratios for xantha₁ and albino characters. The effect of accumulation could be ignored if a representative sample of the natural population could be treated and the number of mutants in the D₁ compared with the natural frequency in a large population. However, it was not possible at the time to handle in the greenhouse the progeny of more than 2000 plants, which totalled more than $\frac{1}{4}$ million individuals. A relatively small sample of 2000 seeds cannot be expected to contain a representative number of accumulated heterozygotes, since their frequency may be only 1 in 50,000. Actually none of the heat-treated seeds were homozygous recessive for any of the mutants studied, and the sample probably did not contain any accumulated heterozygotes, since it was proved in all but one instance (Plant 1340) that only sectors of heat-treated plants were heterozygous. Consequently the number of mutants observed in the D₁ were probably derived solely from mutations occurring in the generation of treatment, while in the large natural population studied the frequency was increased by the accumulation of heterozygotes from many earlier generations. These facts were kept in mind in interpreting the significance of the results obtained.

A field of Wiener's strain of O.A.C. 21 barley, containing $2\frac{3}{4}$ acres, was examined to determine the natural frequency of mutant plants, particularly xantha and albino seedlings which were most easily detected. The total number of plants in the field was estimated by counts of the plants enclosed by a 3 × 3 ft. quadrant allowed to fall at random in 24 scattered areas in the field. Smaller plots of other strains of O.A.C. 21 were also examined. In these plots the total number of plants was estimated by counting a representative number of rows. The results of all these observations are given in Table VII. Xantha and albino mutants were the only types observed. No attempt was made to distinguish xantha₁ from xantha₂ mutants, so that the frequency given in Table VII includes both types. Dwarf and chlorina mutants were never observed. Even if these mutants are more difficult to detect than xantha or albino mutants, it might be significant that none of the former were observed in a population of more than $1\frac{1}{2}$ million whereas one chlorina and three dwarf plants were observed in approximately $\frac{1}{4}$ million D₁ plants after heat treatment.

TABLE VII

FREQUENCY OF OCCURRENCE OF CHLOROPHYLL DEFICIENT MUTANTS IN O.A.C. 21 BARLEY ORIGINALLY OBTAINED FROM VARIOUS SOURCES BUT GROWN AT OTTAWA UNDER NORMAL FIELD CONDITIONS

Original source of strain	Number	Location grown	Approximate no. plants examined	Xantha mutants		Albino mutants	
				No.	Frequency	No.	Frequency
Wiener's strain, Manitoba		D. L. Scott's farm, Ottawa	1,252,000	18	1 in 78,200	66	1 in 19,000
Wiener's strain, Manitoba		Experimental Farm, Ottawa	22,500	0		1	1 in 22,500
Experimental Farm, Indian Head		Experimental Farm, Ottawa	21,700	0		4	1 in 5,400
University of Alberta	Elite 3120	Experimental Farm, Ottawa	23,100	48	1 in 500	3	1 in 7,700
Ont. Agr. College, Guelph	Elite 3099	Experimental Farm, Ottawa	22,500	0		1	1 in 22,500
Macdonald College, Quebec	Elite 3143	Experimental Farm, Ottawa	22,500	0		5	1 in 4,500
Ont. Agr. College, Guelph	C.A.N. 1086	Experimental Farm, Ottawa	22,500	1	1 in 22,500	2	1 in 11,200
Totals			1,386,800	67	1 in 20,700	82	1 in 16,900
Mean frequencies							

The natural frequency of occurrence of xantha₁ and ₂ mutants in Wiener's strain grown at D. L. Scott's farm at Ottawa was 1 in 78,200. The frequency observed in the D₁ generation from Lot 1 of heat-treated material of the same strain was 1 in 13,400. Even without any correction for the accumulation of heterozygotes in nature, it is apparent that the frequency has been raised significantly by the heat treatment. The strain from the University of Alberta contained a very high frequency of xantha plants, 1 in 500. This frequency is far in excess of any other observed in either heat-treated or untreated material. It is probable that this is the result of chance selection of a small sample containing the progeny of a few heterozygous plants. If this population is included in the total, the mean frequency of 1 in 20,700 is still appreciably lower than that observed in the D₁ generation after heat treatment.

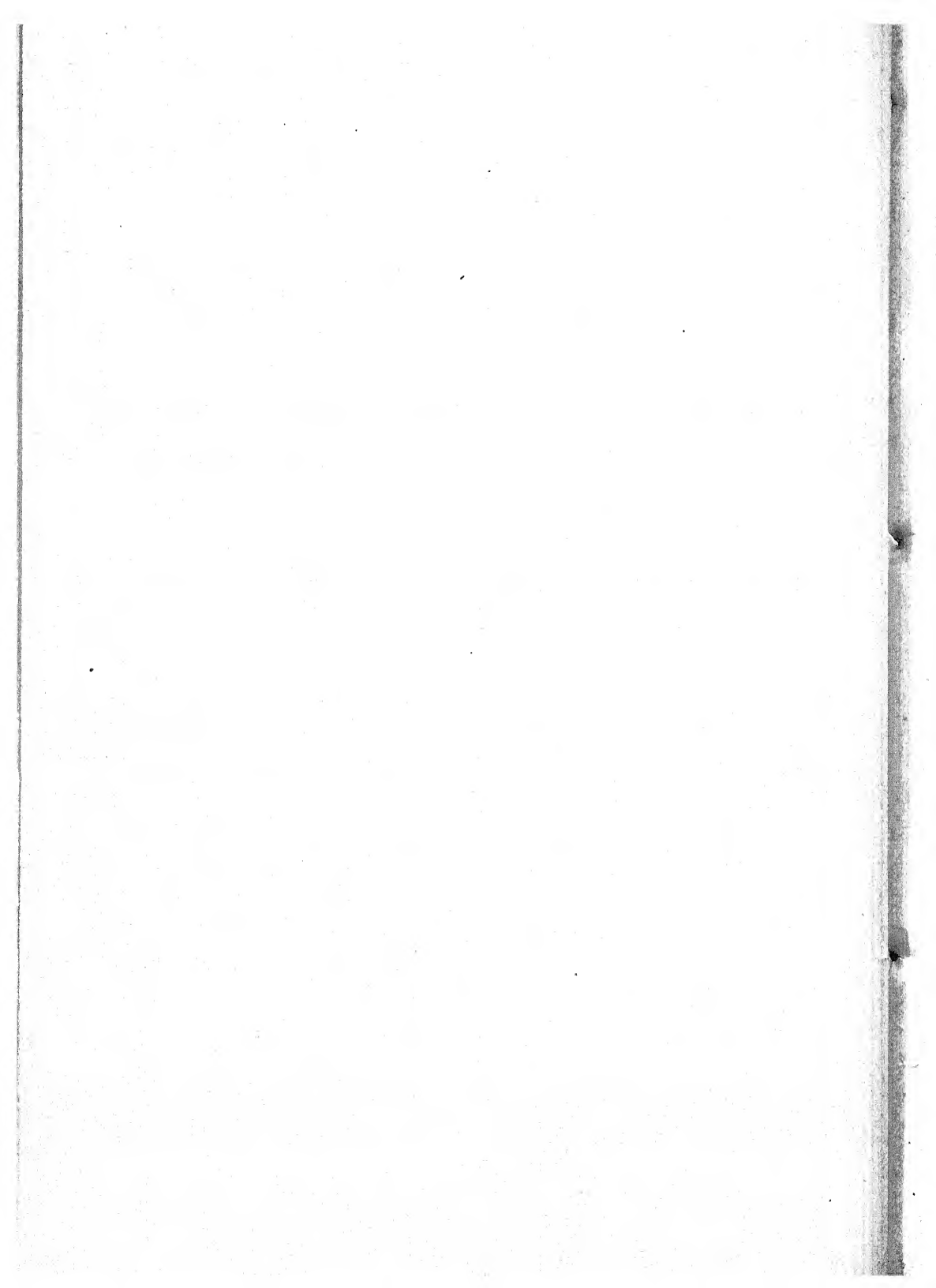
The frequency of occurrence of albinos in the D₁ generation from Lot 1 after heat treatment was 1 in 45,500, whereas the frequency observed in the untreated material of the same strain was 1 in 19,000 or for all the strains examined, 1 in 16,900. Even if a correction was introduced to compensate for the accumulation of heterozygotes in the untreated material, it is apparent that the heat treatment did not increase the mutation rate for the albino character.

Acknowledgments

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EFFICIENCY IN FIELD TRIALS OF PSEUDO-FACTORIAL AND INCOMPLETE RANDOMIZED BLOCK METHODS¹

By C. H. GOULDEN²

Abstract

Uniformity data for eight different crops were studied with the object of making comparisons of the efficiency of Incomplete and Randomized Block methods. Altogether 26 different comparisons were made.

In general the Incomplete Block method gives increases in efficiency, such increases being partially correlated with soil heterogeneity. If the field is very uniform there may be a loss in efficiency but this is rather unlikely on the average field and with careful planning of the experiment. The increases in efficiency due to the use of Incomplete Block methods would appear to vary on the average from 20 to 50%. In view of the greater adaptability of these methods to irregularly shaped fields, in addition to greater efficiency, their use can be generally recommended.

The relative efficiency of Incomplete and Complete Block methods was studied in relation to the size and shape of plots and blocks. The former method gives the greatest gains in efficiency when the Incomplete Blocks are nearly square and are made up of long narrow plots.

Introduction

For field tests involving a large number of varieties Yates (7, 8) has proposed the Pseudo-factorial and Incomplete Randomized Block methods. The Pseudo-factorial method in particular is recommended owing to its greater adaptability with respect to the number of replications. From a study of uniformity data Yates (7) indicates that, if there is a sufficient degree of heterogeneity in the field, gains in efficiency by using the Pseudo-factorial method as compared to ordinary Randomized Blocks may range from about 20 to 50%. In the present paper results are given of studies with uniformity data from field experiments on the relative efficiency of Pseudo-factorial and Incomplete Blocks, with special reference to the degree of soil heterogeneity and to the size and shape of the plots and blocks.

It is a well known fact that compact plots or blocks of land are more variable than long narrow strips, although in the case of long narrow strips this only applies to strips that are placed side by side in the field. Therefore we must arrange field experiments so that the units designed for error control are as compact as possible and the units designed for comparing varieties or treatments within the error control units are as long and narrow as possible. Thus in a Randomized Block experiment the ideal arrangement is to have square blocks and each block divided into the required number of strips to be used

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as plots. Owing to practical considerations that limit plot shape this is not always possible, but we must always keep this principle in mind in designing an experiment, so as to approach as nearly as we can to the ideal situation.

In general, plot shapes vary from 1 : 5 to 1 : 20, and consequently groups of plots varying from 5 to 20 can be placed in square blocks. Therefore in designing an Incomplete Block experiment (for convenience including in this terminology both types mentioned above) it is generally much easier to make compact blocks than it is in the case of Randomized Blocks. Now in a study of uniformity data, in order to make a comparison of Incomplete and Randomized Block experiments, it is necessary to make both kinds of blocks as compact as possible to be fair to both arrangements; but to a certain extent this is unfair to the Incomplete Block design which has great adaptability to fields of various shapes and may give error control on fields where Randomized Blocks would be relatively inefficient. This is particularly true of fields that are very irregular or long and narrow. Furthermore in considering any particular set of uniformity data it may be obvious that a given type of Incomplete Block experiment will give the best results, but if this type cannot be compared with a Randomized Block experiment on the same field, then another type of Incomplete Block experiment, which is probably less efficient, must be used.

The procedure for making comparisons of the efficiency of the two methods is as illustrated in Fig. 1 and Table I. Fig. 1 is an outline to scale of a field of orange trees, the data from which were published by Batchelor and Reed (1). In this case each tree is taken as a single plot, and since the trees were the same distance apart in the rows and columns, the plots may be taken to be square. The portion of the field taken contains 810 trees and we can assume that it

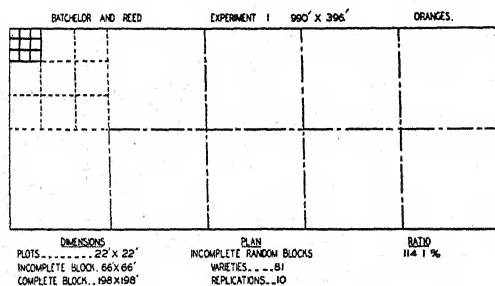


FIG. 1.

TABLE I*

ILLUSTRATING METHOD OF MAKING TESTS OF THE EFFICIENCY OF INCOMPLETE BLOCKS AS COMPARED TO RANDOMIZED BLOCK EXPERIMENTS

		SS	D F	M S
Incomplete Blocks	Blocks	1,004,655	89	11288
	Error	857,682	720	11912
	Total	1,862,337	809	
Complete Blocks	Blocks	654,078	9	72675
	Error	1,208,259	800	15103
	Total	1,862,337	809	

$$\text{Ratio} = \frac{15103}{11912} \times \frac{9}{10} = 114.1\%.$$

*Data correspond to Fig. 1.

is being used to test 81 varieties in 10 replications. In the figure the dotted lines represent the Randomized Blocks that might be used. They are designated here as Complete Blocks in contra-distinction to Incomplete Blocks. One Complete Block is shown divided into Incomplete Blocks and one of the latter is shown divided into plots. The dimensions of all of the units can then be seen in relation to each other. Here we have square plots and square blocks of both kinds. The arrangement of the square plots in square blocks is obviously the most desirable from the standpoint of error control. The test of efficiency is made by first carrying out a simple analysis of variance for each design as in Table I. We note that the Incomplete Blocks result in a lower error variance; but this is not pure gain in efficiency, as in comparing two varieties by this method the variance of a mean difference as ordinarily computed must be multiplied by an efficiency factor arising from the particular type of Incomplete Block experiment used. Here the efficiency factor, which is explained in more detail below, is 9/10. Where the error mean square arising from the Incomplete Block experiment is error (*i*) and that from the Complete Block experiment is error (*c*) the relative efficiency in percentage of the former is given by the ratio—

$$\frac{\text{Error } (c)}{\text{Error } (i)} \times \text{Efficiency Factor} \times 100.$$

Thus if our ratio is 120%, the gain in efficiency due to using Incomplete Blocks is 20%.

The Efficiency Factor

The efficiency factor of Incomplete Block experiments must be considered in some detail in making efficiency tests, as in certain cases an Incomplete Block experiment may be considered as belonging to one of several types, and one has to decide whether it is justifiable to use the type having the highest efficiency factor. Thus a uniformity experiment assuming 64 varieties to be tested in Incomplete Blocks of eight plots, and with six replications, may be considered as a simple Two Dimensional Pseudo-factorial with two groups of sets, or as a Two Dimensional Pseudo-factorial with six groups of sets, one for each replication. In the first case the calculation of the corrected variety means and the variety sum of squares is relatively simple but in the second case the calculations are somewhat involved. The method of carrying out the calculations in the second case has apparently not yet been published but it is merely an extension of the method given by Yates (7) for Two Dimensional Pseudo-factorials with three groups of sets. In the first case the efficiency factor is $9/11 = 0.8182$, and in the second case it is $45/51 = 0.8824$. In considering exactly the same uniformity data, therefore, the greatest efficiency can be obtained by assuming the experiment to be one with six groups of sets. This method would probably not be used in actual practice, but it would seem to be justifiable to consider the theoretical experiment as such owing to the fact that the reason we have only six replications is that the data are limited. In designing an actual experiment we would probably endeavor to use nine replications, in which case we would have an Incomplete Block

experiment of the symmetrical type for which the calculations are quite simple, and for which the efficiency factor has a maximum value of 9/10.

The efficiency factor for any Incomplete Block experiment of the Two Dimensional type is given by—

$$E = \frac{(k-1)(p+1)}{(k-1)(p+1) + k}$$

where k represents the number of groups of sets and p is the number of plots in an Incomplete Block. This formula does not hold for the symmetrical type of experiment for which the efficiency factor is always $\frac{p}{p+1}$.

The changes in the efficiency factors for different values of k and p are illustrated in Fig. 16 for $k = 2$ to 11 and $p = 4$ to 12. An important feature of this graph is that for the higher values of p the increase in the efficiency factor is very small after we pass $k = 6$ or 7. However even for $k = 5$ the computations in actual practice are quite cumbersome, so that the experimenter will rarely ever go beyond $k = 4$ unless he can go to the limit, whereupon the computations very suddenly become simplified. Of course in certain cases, such as in a test of 36 varieties, it is impossible to go beyond $k = 3$ and therefore the Pseudo-factorial method is the only one available. In general the difference between $k = 2$ and $k = 3$ is quite marked and consequently it would seem advisable where reasonably good computational facilities are available to use the three groups of sets.

Results of Efficiency Tests

The results of the efficiency tests are presented in Figs. 1 to 15, which are all of a type similar to Fig. 1 which has been described. Since the results of such tests are only of interest in relation to the particular layout considered, this method of presentation has been followed throughout. The method of investigation was to take a set of uniformity data and arrange comparisons of Incomplete and Randomized Block experiments in different ways. For example the data published by Sayer (4) consists of yields of sugar cane plots measuring 3×60 ft. As illustrated in Fig. 5, the first experiment consisted of Incomplete Blocks measuring 24×60 ft. and Complete Blocks measuring 120×196 ft. The latter were therefore more compact than the former, and as a result, the gain in efficiency due to the use of Incomplete Blocks is only 7.3%. In Experiment II the Incomplete Block is the most compact and the effect of this is shown in the efficiency gain of 15%. Finally as in Experiment III the Complete Blocks are again the most compact but the original plots have been combined in fours and a great deal of the irregular soil variability within Incomplete Blocks has been removed. The result is a gain in efficiency of 37%. The combining of plots in order to remove minor soil variations from within the blocks seems in general to be profitable, and would appear to be due to the smoothing out of variations due to direct accidents to individual plots such as weed growth, cracking of the soil, variations in stand, and so forth. These are rather different from other factors

affecting soil variability, such as soil texture, moisture supply, and general fertility. When the minor factors are largely overcome, the plot yields tend to reflect true patchiness effects and fertility trends. It is these variability factors that are removed by the Incomplete Block method and, consequently, the best results are obtained when the minor variability factors are largely eliminated.

The series of orange-tree yields given by Batchelor and Reed are of especial interest in relation to the efficiency of the Incomplete Block method. The plots are single-tree yields, each tree occupying an area 22 feet square. In Fig. 1, the original plots are shown combined in square Incomplete and square Complete Blocks. The efficiency test is therefore perfectly balanced with respect to the two methods. The gain due to the Incomplete Blocks is 14.1% and the reason that this is not a large gain is probably the fact that square plots, although arranged as compactly as possible in the blocks, are not particularly efficient. In Fig. 3 the plots are shown combined in twos, and while the Incomplete Blocks are not as compact as the Complete Blocks, the gain in efficiency is now 25.5%. In Fig. 4 the plots have been combined in fives and now represent reasonably efficient units. The Incomplete Blocks are still not as compact as the Complete Blocks but the gain is now 47.7%.

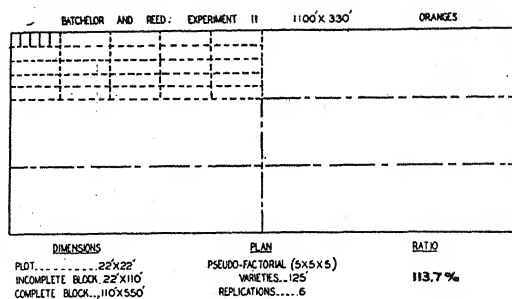


FIG. 2.

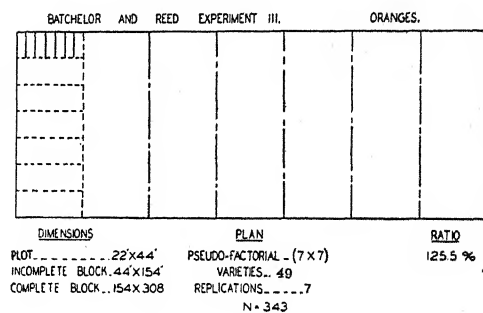


FIG. 3.

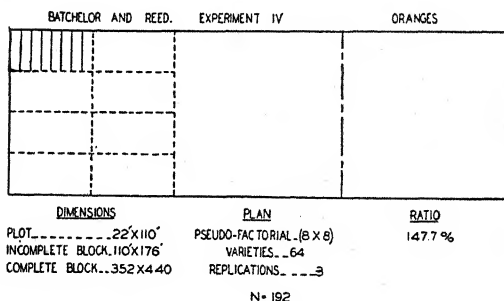


FIG. 4.

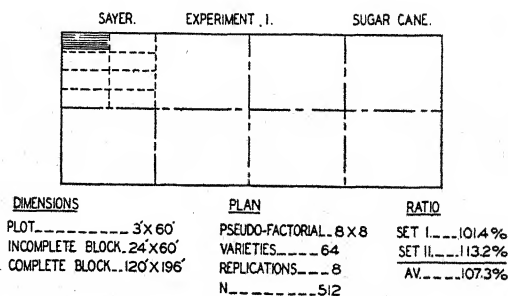


FIG. 5.

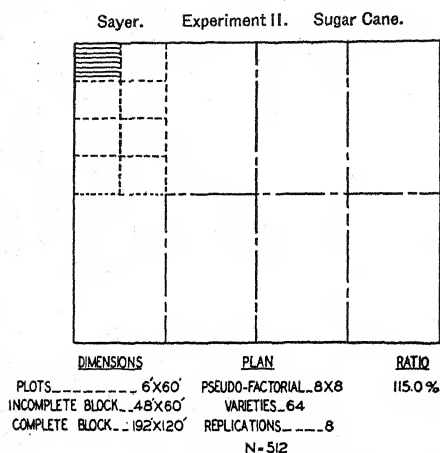


FIG. 6.

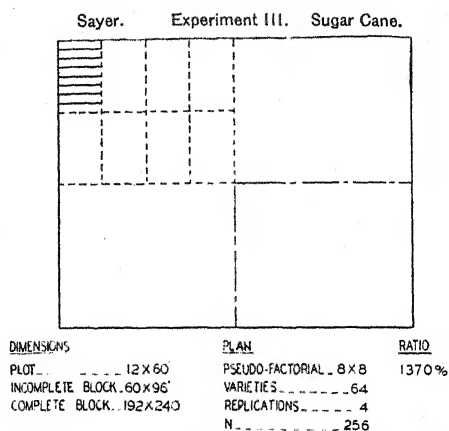


FIG. 7.

It should be made clear at this point that the change in plot shape is considered only in relation to the relative efficiency of Complete and Incomplete Block experiments. For example in the case cited above it is not argued that the plots combined in fives are more efficient than if in the same area the original square plots are used with five times as many replications. In actual practice the experimenter will have to balance up the two factors, namely, the increase in efficiency due to making the Incomplete Blocks compact and the increases in efficiency due to greater replication with less efficient blocks. For plots of equal area, however, the above results apply directly, since the number of replications will remain the same; and in general the assumption may be made, for example, that if the square plots in Batchelor and Reed Experiment I were of the same area as those in Experiment IV the increase in efficiency would not be as great as in the latter case.

Fig. 2 is an illustration, with the Batchelor and Reed data, of a Three Dimensional Pseudo-factorial experiment. The varieties are assumed to number 125 and these are tested in blocks of five plots using three groups of sets. The gain in efficiency of 13.7% is quite satisfactory, considering that the Incomplete Blocks are not very compact and the original single plot yields are used. If sufficient plots were available to allow for combining them in fours, a much greater gain in efficiency would be expected.

The experiments with the square yard barley yields illustrated in Figs. 8 and 9 form a series very similar to those for the Batchelor and Reed plots except that only two good arrangements were possible. The square plots do not give any increase in efficiency, in fact there is a loss of 7.1%. When combined in eights to form plots 1 yd. x 8 yd. the increase in efficiency is 17.9%, and this may be considered reasonably satisfactory. Fig. 10 illustrates an experiment in which a deliberate attempt was made to obtain a reduction in efficiency by making the Incomplete Blocks long and narrow and the Complete Blocks compact. The result was a reduction in efficiency of 37.2%,

and illustrates very clearly that success with the use of Incomplete Blocks does not arise from the method in itself but from the fact that it allows for the planning of an experiment in such a way as to give real increases in efficiency.

The results with potato yields from Kirk and Goulden (3) is an example of a field which does not permit of a comparison using compact Complete Blocks. The yields given were actually for varieties in a Randomized Block experiment and therefore the yield of each plot was corrected for the variety effect. As would be expected, the results are erratic. Two sets of data were available, one set giving practically no increase in efficiency while the other set gave an increase of 183.1%. This result may be used as an illustration of the adaptability of the Incomplete Block method to fields of varying shape. To use Complete Blocks of the type illustrated in Fig. 11, on account of irregularities or roadways in the field, would obviously give on the whole very bad results. However, on any such field the Incomplete Blocks would allow the setting up of an efficient design.

The results for the data by Summerby (5) illustrate again the unpracticability of square plots, especially when they are combined in Incomplete Blocks that are long and narrow. Throughout the seven sets studied the plan was the same, the Incomplete Blocks being narrow and the Complete Blocks square. In view of this fact it is remarkable that the efficiency ratios average 100.8%. In spite of the poor layout there was on the average no loss in efficiency.

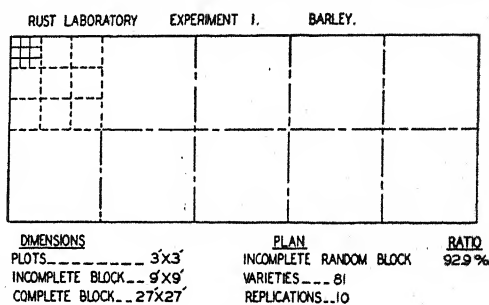


FIG. 8.

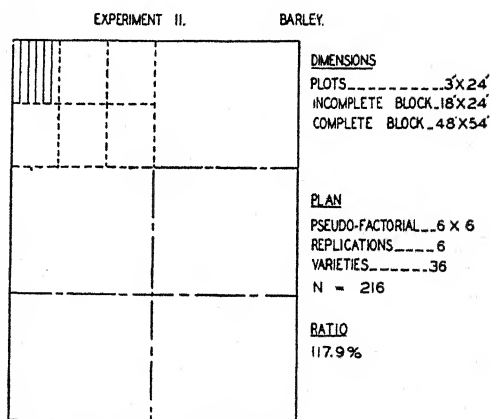


FIG. 9.

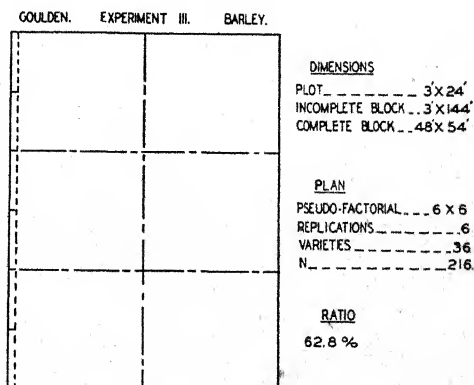


FIG. 10.

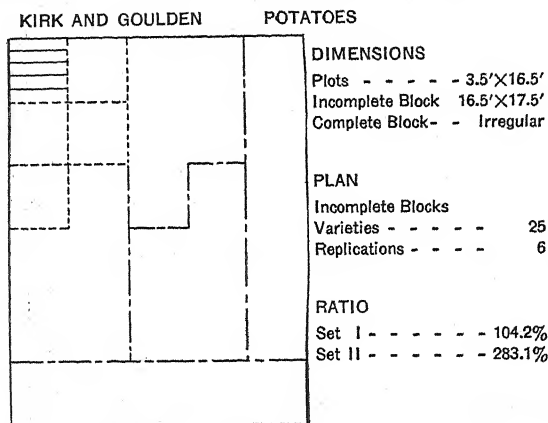


FIG. 11.

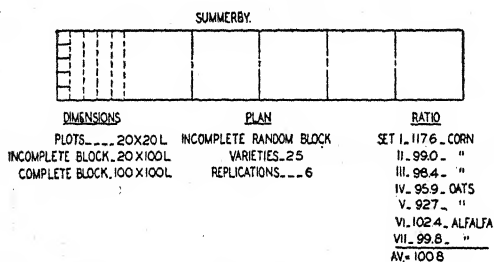


FIG. 12.

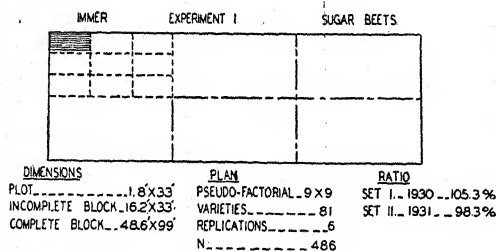


FIG. 13.

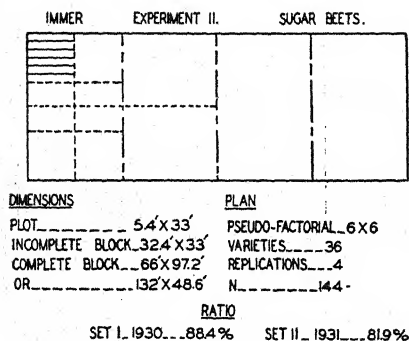


FIG. 14.

The sugar beet plots came from one set unpublished but furnished by the courtesy of Dr. F. R. Immer and another set from a similar nearby field on which certain results have already been given by Immer (2). These plots are of interest in that, in spite of a favorable plan for the assumed Incomplete Block experiment in both Experiments I and II, the first gave an average ratio, for the two sets, of only 101.8%, and the second of only 85.2%. This field appears to be exceptionally uniform, and in such cases it does appear that there is a decided possibility of a loss in efficiency through using the Incomplete Block method. It would be of interest here, however, to study in further detail the causes of plot variability. If variability from plot to plot is caused by variations in stand and other factors of that nature it might easily be that the Incomplete Block method would be incapable of bringing about any improvement in efficiency.

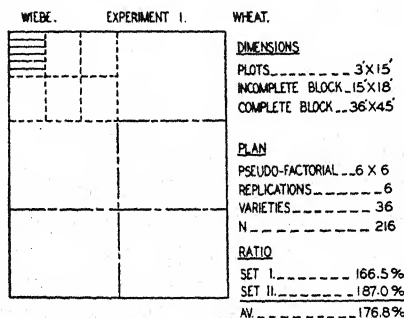


FIG. 15.

The results for the data by Wiebe (6) are in direct contrast to those for the data by Immer. In the former, the plots show a decided variability and this is reflected largely in patchy effects and fertility gradients. The plots were first combined in threes but still provided a sufficient number of plots to give two sets of data for which efficiency ratios could be calculated as indicated in Fig. 15. The average efficiency ratio was 176.8%. This result

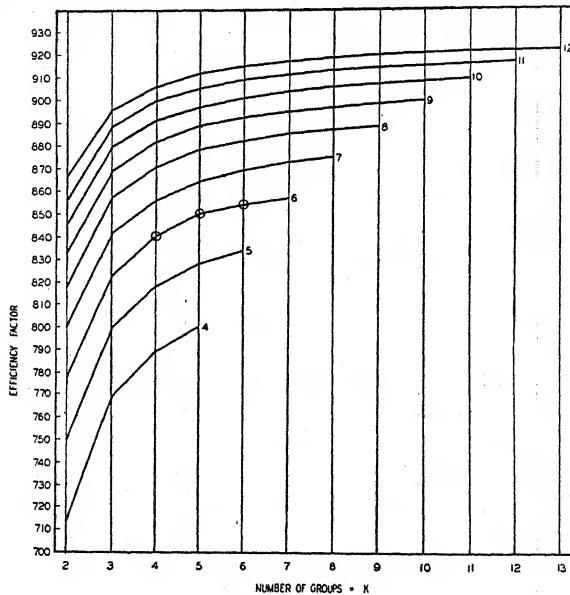


FIG. 16. The changes in the efficiency factor for $k = 2$ to 11, and $p = 4$ to 12. The points covered by small circles are impossible owing to the impossibility of forming a completely orthogonalized (6×6) square.

is particularly important in that a great many yield trials of wheat varieties are conducted in plots of approximately the same shape as in this study. The increase in efficiency of about 77%, if general throughout such trials, would be extremely important.

From the data studied it is very difficult to come to any general conclusion as to the increase in efficiency to be expected from the use of the Incomplete Block methods. Under average conditions and with careful planning of the experiment, increases in efficiency ranging from 20 to 50% would appear to be possible. In certain cases, with very uniform fields, there may be a slight loss in efficiency, but such fields are probably rare. With very patchy fields on which experimenters frequently have to work, the gain in efficiency may easily exceed 50% and may be as great as 100%.

The reason for the increased efficiency of Incomplete Block as compared to Randomized Block field trials is to be found obviously in the relative efficiency for error control being sufficiently great to more than overcome the efficiency factor. The point of relative efficiency in error control of the

two types of blocks is rather important, as it might be assumed that the Incomplete Blocks would always give higher efficiency with increasing heterogeneity. That this is not the case is obvious from Table II which gives for each of the cases investigated the efficiency ratio, the intra-class correlation (r_i) for the Incomplete Blocks and the intra-class correlation (r_c) for the

TABLE II

EFFICIENCY RATIOS FOR ALL SETS OF DATA STUDIED AND THE CORRESPONDING INTRA-CLASS CORRELATION COEFFICIENTS FOR THE INCOMPLETE AND COMPLETE BLOCKS

—	Exp.	Set	Ratio	r_i	r_c	$r_i - r_c^2$
Rust Laboratory	III		62.8	0.2666	0.4470	0.067
Immer	II	2	81.9	.0472	— .0066	.047
Immer	II	1	88.4	.1382	.0210	.138
Summerby	I	5	92.7	.5546	.5409	.262
Rust Laboratory	I		92.9	.0738	.0475	.072
Summerby	I	4	95.9	.2479	.1498	.225
Immer	I	2	98.3	.0930	— .0005	.093
Summerby	I	3	98.4	.3883	.3058	.295
Summerby	I	2	99.0	.3782	.2886	.295
Summerby	I	7	99.8	.7324	.7103	.228
Kirk	I	1	101.4	.6127	.5646	.294
Sayer	I	1	101.4	.0180	.0687	.013
Summerby	I	6	102.4	.7027	.6678	.257
Immer	I	1	105.3	.2038	.0386	.202
Sayer	I	2	113.2	.4255	.2892	.342
Batchelor, Reed	II		113.7	.5645	.3513	.441
Batchelor, Reed	I		114.1	.4850	.3678	.350
Sayer	II		115.0	.3801	.2138	.334
Summerby	I	1	117.6	.4932	.3120	.396
Rust Laboratory	II		117.9	.6122	.4470	.412
Batchelor, Reed	III		125.5	.6307	.5006	.380
Sayer	III		137.0	.4575	.2380	.401
Batchelor, Reed	IV		147.7	.6789	.5323	.396
Wiebe	I	1	166.5	.7529	.5058	.497
Wiebe	I	2	187.0	.7151	.3420	.598
Kirk	I	2	275.3	.8377	.4953	.592

Complete Blocks. Since the Incomplete Blocks are relatively small units, the corresponding intra-class correlation may be taken as a general measure of soil heterogeneity. There is a certain degree of correlation between this measure and the efficiency ratio in that we must have fairly high values of r_i before high efficiency ratios can be obtained, but in certain cases of high values of r_i the efficiency ratio is low. This arises from the fact that in these cases r_c is also high. As examples of this fact we may take the two cases as given in Table II for Summerby Experiment I, Set 7, and Sayer Experiment III. In the first we have $r_i = 0.7324$, and $r_c = 0.7103$. In the second we have $r_i = 0.4575$ and $r_c = 0.2380$. An arbitrary constant which seems from a preliminary examination of the data to be reasonably well correlated with the efficiency ratio is ($r_i - r_c^2$). This is given in the last column of Table II.

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LABORATORY MALTING. II. PRECISION¹

BY J. ANSEL ANDERSON² AND W. O. S. MEREDITH³

Abstract

The precision of the malting test made in equipment already described (Can. J. Research, C, 15 : 204-216. 1937) was studied by making four batches of malt each of which contained duplicate malts made by eight treatments representing the combination of steeping for 48 or 60 hr., maintaining the germination chamber at 53° or 54.5° F., and kilning for 36 hr. at 100° to 175° F. or for 52 hr. at 90° to 165° F. The standard errors of duplicate tests made in the same and in different batches were found to be: extract, 0.08 and 0.09%; moisture, 0.04 and 0.05%; color, 0.04 and 0.05 units; diastatic power 1.0 and 2.8° L.; permanently soluble nitrogen as percentage of wort solids, 0.01 and 0.02%; malting loss 0.06 and 0.29%; and sprouts, 0.06 and 0.18%.

On the average, increasing the time of steeping decreased extract by 0.08%; but increased diastatic power by 3.2° L., permanently soluble nitrogen by 0.05%, malting loss by 0.98% and sprouts by 0.44%. Increasing the temperature of the germination chamber increased diastatic power by 4.2° L., permanently soluble nitrogen by 0.04%, malting loss by 0.93%, and sprouts by 0.52%. Increasing the time and decreasing the temperature of kilning increased extract by 0.08% and diastatic power by 14.8° L. Statistical analyses show that the test is sufficiently precise to prove that these effects, though small, are significant.

It is apparent that the usefulness of a laboratory malting test as a research tool will be limited by the precision of the test. The greater the precision, *i.e.*, the more nearly it is possible to reproduce identical malts from the same barley, the less will be the replication required to prove whether significant differences exist between samples of very similar malting qualities, the finer will be the differentiation between samples, and the greater will be the ease with which the effects of variety and environmental conditions on malting quality can be demonstrated. For these reasons, and because comparisons based on methods of unknown precision lose much of their value, it would appear that it is the first duty of any new malting laboratory to determine the level of precision of its malting test.

The investigation reported in this paper was undertaken primarily with the object of determining the level of precision of the malting test developed in the National Research Laboratories during the past year and the scope of the work which could be undertaken with it. It also serves to demonstrate the design of investigation for which the malting equipment is particularly suited, namely, a factorial design involving the simultaneous study of three factors, each of which is tested at two levels.

Design of Investigation

The investigation was made with equipment which consists of duplicate sets of steep tank, germination chamber and kiln, each set having a capacity of eight 350-gm. samples (2). Four batches of 16 malts were made under as

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nearly identical conditions as the precision of control in the equipment would permit. In each batch, eight samples were steeped in each tank, and four samples from each tank were then put into each germination chamber. At the end of the germination period there were thus four sets of quadruplicate samples, each set having been exposed to a different combination

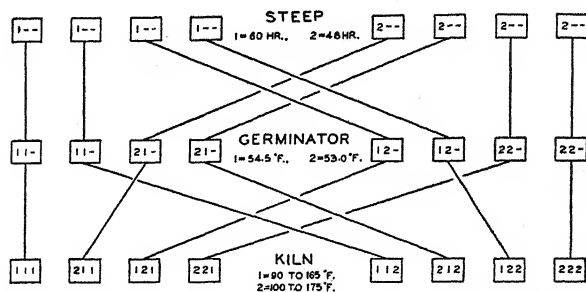


FIGURE 1. Factorial distribution of samples. First digit = steep; second digit = germination; third digit = kiln.

of steeping and germination conditions. Two samples from each of these four sets were then placed in each kiln; thus after kilning, there were eight sets of duplicate samples, each of which had been exposed to a different combination of malting conditions. The eight treatments will hereafter be designated by numbers of three digits, the first representing the steeping procedure, the second the germination procedure and the third the kilning procedure (Fig. 1).

Steeping

Methods

In Procedure 1 the samples were steeped for 60 hr. at 50° F., in Procedure 2 the time was reduced to 48 hr.

The samples were steeped for the first 48 or 36 hr. in quart sealers, and for the last 12 hr. they were steeped in 8-mesh cages. During the first period, the water was decanted and the samples were allowed to remain without water for 1 hr. at the end of each 12 hr.

Germination

In Procedure 1 the chamber was maintained at 54.5° F. and in Procedure 2 the chamber was maintained at 53.0° F.

After being in the chamber for 8 hr. in 8-mesh cages the samples were transferred to galvanized iron cylindrical cans with eight $\frac{1}{8}$ -in. holes. At 120 hr. the samples were transferred back to 8-mesh cages to permit some withering.

All samples were rotated continuously at 0.05 r.p.m. and were treated with 15 ml. of water at 72 hr.

Kilning

Procedure 1 consisted of kilning for 52 hr. at temperatures of 90° to 165° F. and Procedure 2 of kilning for 36 hr. at temperatures of 100° to 175° F.

The details were as follows:— No. 1, 8 hr. at 90° F., 18 hr. at 120° F., 22 hr. at 135° F., and 4 hr. at 165° F.; No. 2, 8 hr. at 100° F., 18 hr. at 130° F., 6 hr. at 145° F., and 4 hr. at 175° F. The samples were held in 8-mesh cages and were rotated continuously at 0.8 r.p.m.

General

In order to make sure that differences between the malts were the result of the controlled differences in malting procedure, and not of uncontrolled idiosyncrasies of any unit of the equipment, the procedures were used alternately in each set of equipment.

Methods for Malting Loss and Sprouts

Immediately after kilning the malt was kneaded in a small bag. The sprouts which were rubbed off during this process were separated by sifting and weighed to the nearest 0.1 gm. The polished malt was weighed to the nearest 0.5 gm. Malting loss and sprouts are reported as grams per 100 gm. of barley dry matter.

Analytical Methods

Each malt was divided into duplicate samples with a Boerner sampler. The samples were then analyzed in random order for extract, moisture, color, diastatic power and permanently soluble nitrogen.

Extract (fine grind) and moisture were determined by the Official Methods of the American Society of Brewing Chemists (1). Color was determined by the Official Method except that a Lovibond tintometer, made by the British Drug Houses, Limited, was used.

Diastatic power was determined by the modification of the Official Method proposed by Anderson and Sallans (3).

The permanently soluble nitrogen in the wort was determined by adding 10 ml. of a buffer solution, containing equal parts of normal acetic acid and normal sodium acetate solutions to 50 ml. of wort, heating for 30 min. in a boiling water bath, filtering, transferring 25 ml. of the filtrate to a Kjeldahl flask, evaporating to a thick syrup on the steam bath, and determining the nitrogen by the Kjeldahl method. Permanently soluble nitrogen is reported as a percentage of total wort solids.

Materials

A bulk lot of O.A.C. 21 barley, having a nitrogen content of 2.06% and a 1000-kernel weight of 32.1 gm., was split with a Boerner sampler into 64 samples each representing 300 gm. of barley dry matter. The samples were malted in random order.

Experimental Results

The experimental data are too numerous to permit publication of all of them, but excerpts from them and various summaries are presented in Tables I to V. A non-statistical discussion of results is presented in the next section, which is followed by a short section giving the results of the statistical analyses.

Discussion

Precision of Malting Tests made in the Same Batch

The precision of comparisons of samples that are malted in the same batch is affected by two errors, that of malting and that of analysis. Information on both errors is provided by the investigation as each batch contained eight sets of duplicate malts and each malt was subjected to duplicate analyses. The complete data for extract and diastatic power from Batch 1 are presented in Table I in the form of differences between duplicate malting tests and between duplicate analyses. It will be observed that the former are of little greater magnitude than the latter.

TABLE I
COMPLETE DATA FOR BATCH I FOR EXTRACT AND DIASTATIC POWER IN TERMS OF DIFFERENCES
BETWEEN DUPLICATE MALTING TESTS AND BETWEEN DUPLICATE ANALYSES

Treatment	Extract, %		Diastatic power, °L.	
	Duplicate malts	Duplicate analyses	Duplicate malts	Duplicate analyses
111	.40	.12 .09	1.6	0.0 3.7
112	.30	.09 .05	1.7	0.1 0.2
121	.13	.22 .12	0.4	0.2 3.8
122	.06	.15 .03	0.8	0.8 0.8
211	.22	.13 .21	1.6	0.0 0.6
212	.06	.22 .11	0.3	3.3 3.4
221	.18	.11 .04	4.8	1.8 2.9
222	.16	.07 .23	0.6	1.3 0.3

A more concise picture of the errors can be obtained by presenting them in the form of standard errors. These have been calculated for all determinations from the complete data of the investigation and are reported in the first two columns of Table II. Since both malting tests and analyses will generally be made in duplicate, it seemed best to report the standard errors for means of duplicate tests and analyses.

The data show that the standard errors for tests made in the same batch are of about the same magnitude as those for analyses. It must be concluded, therefore, that the errors of malting are comparatively small and are masked by the errors of analysis, even though these are also small. For diastatic

TABLE II

STANDARD ERRORS FOR THE MEANS OF DUPLICATE ANALYSES AND DUPLICATE MALTING TESTS
MADE IN THE SAME AND DIFFERENT BATCHES

Determination	Duplicate analyses	Duplicate malting tests	
		In same batch	In different batches
Extract, %	.07	.082	.093
Moisture, %	.038	.037	.045
Color, Lovibond units	.049	.043	.049
Diastatic power, °L.	.80	.99	2.77
Permanently soluble nitrogen as % of wort solids	.016	.011	.024
Malting loss, %	—	.065	.292
Sprouts, %	—	.058	.179

power and extract, the standard errors for tests are larger than those for analysis, but statistical analyses showed that the difference did not attain a 5% level of significance. In other words, the available data fail to prove that the variation between duplicate tests cannot be accounted for by chance combinations of variations in analytical results. It appears that the level of precision attained can be considered satisfactory and that if improvement is desired it will be necessary first to increase the precision of the analyses.

Precision of Malting Tests Made in Different Batches

In any but very small investigations it will be impossible to base all comparisons on tests made in the same batch. It is therefore necessary to consider the precision of tests made in different batches. This will be affected by an additional error which represents the variations in malting conditions between batches, which are the result either of personal errors or of failure of the equipment to maintain exactly the same set of conditions over extended periods.

The standard errors for the means of duplicate tests made in different batches are reported in the last column of Table II. Since only four batches of malt were made, the data must be considered only as a rough estimate of the errors. It is apparent that the variation between batches adds very considerably to the error of the test.

Further information on the variation between batches can be obtained from Table III. The data represent the means, over all treatments, for each determination and for each of the four batches. It is evident that the results for Batch 4 are considerably higher than those for the other three batches. The malting records show that the temperature controls for the steep tanks failed to function properly when Batch 4 was being made, owing, apparently, to a sudden increase in room temperature during a spell of mild weather. The temperature of the steeping water was more than 2° F. too high for part of the time.

TABLE III

MEANS, OVER ALL TREATMENTS, FOR EACH DETERMINATION AND EACH BATCH

Determination	Batch 1	Batch 2	Batch 3	Batch 4	Range
Extract, %	75.25	75.20	75.22	75.36	0.16
Moisture, %	3.63	3.68	3.62	3.69	0.07
Color, Lovibond units	2.13	2.17	2.12	2.20	0.08
Diastatic power, °L.	128.5	130.6	127.4	136.0	9.2
Permanently soluble nitrogen as % of wort solids	1.46	1.46	1.43	1.50	0.07
Malting loss, %	10.73	10.85	10.41	11.38	0.97
Sprouts, %	4.35	4.47	4.32	4.85	0.53

On the whole, it appears that duplicate tests made in different batches attain a level of precision which is adequate for most practical purposes. Nevertheless, for some investigations a further improvement may be desirable. The problem of decreasing the variation between batches is presumably that of obtaining more precise control of conditions in the equipment, particularly with respect to long periods of time. Investigations looking towards the solution of this problem are now being undertaken.

It should be noted that the malting test may be subject to an additional error about which the present investigation yields no information. Harrison and Rowland (5) proposed that all samples be steeped for the same length of time but Dickson *et al.* (4) steep all samples to the same moisture content, determining the time required by pilot steeping tests. If the latter procedure is preferable, which it appears to be, then the accuracy with which the set moisture content can be attained in duplicate samples will affect the precision of the malting test. This consideration did not come in question in the present investigation in which only samples of the same lot of barley were used.

Effects of Different Treatments

The treatments studied consist of the eight possible combinations of steeping for 48 or 60 hr., germinating with the chamber at 53° or 54.5° F., and kilning for 52 hr. at 90° to 165° F. or for 36 hr. at 100° to 175° F. The investigation was not undertaken with the object of studying the effects of different treatments on the resulting malt, since information on these is already available (6) and any experienced maltster could have predicted the qualitative if not the quantitative results. Moreover, general conclusions could hardly be based on an investigation made with only one sample of barley. The study was made primarily to determine the capacity of the equipment and method, so far developed, for proving that differences in procedure affect the resulting malt, and particularly their capacity for proving that a change at one stage of the procedure will have different effects depending upon how other parts of the procedure are carried out.

The data for all series are presented in Table IV in mean values for each treatment over all batches. Each figure, therefore, represents the mean of duplicate determinations made on eight malts.

TABLE IV
MEANS, OVER ALL BATCHES, FOR EACH TREATMENT

Determination	Treatments							
	111	112	121	122	211	212	221	222
Extract, %	75.21	75.14	75.27	75.26	75.37	75.22	75.34	75.26
Moisture, %	3.69	3.52	3.72	3.59	3.75	3.57	3.74	3.63
Color, Lovibond units	2.07	2.28	2.04	2.28	2.07	2.30	2.02	2.19
Diastatic power, °L.	142.1	126.8	137.8	122.9	139.2	123.4	133.6	120.4
Permanently soluble nitrogen as % of wort solids	1.50	1.51	1.46	1.47	1.46	1.47	1.42	1.42
Malting loss, %	11.91	11.91	10.79	10.72	10.78	10.62	10.09	9.92
Sprouts, %	5.02	4.95	4.43	4.47	4.58	4.48	4.03	4.02

A better comparison of the effects of the changes in procedure can be obtained by rearranging and condensing the data. The treatments can be arranged in four pairs differing only in steeping procedure (111 and 211, 112 and 212, 121 and 221, 122 and 222), in four pairs differing only in germination procedure, (111 and 121, etc.), or in four pairs differing only in kilning procedure (111 and 112, etc.). From these data, the mean effects, over all treatments, of changes in steeping, germination and kilning procedure, can be calculated as differences between means of four treatments. Data representing these differences are presented in Table V. Those differences which were shown to be significant by statistical analyses have been marked with an asterisk.

TABLE V
DIFFERENCES CAUSED BY CHANGE IN STEEPING, GERMINATION AND KILNING
PROCEDURES 1 AND 2

Determination	Steeping, 1 minus 2	Germination, 1 minus 2	Kilning, 1 minus 2
Extract, %	-0.08*	-0.04	0.03*
Moisture, %	-0.04	-0.04	0.15*
Color, Lovibond units	0.02	0.05	-0.21*
Diastatic power, °L.	3.2*	4.2*	14.8*
Permanently soluble nitrogen as % of wort solids	0.05*	0.04*	-0.01
Malting loss, %	0.98*	0.93*	0.09
Sprouts, %	0.44*	0.52*	0.03

* Statistically significant.

The data show that extract is not very sensitive to changes at any stage of the procedure; that moisture and color are sensitive only to changes in kilning; that diastatic power and permanently soluble nitrogen are fairly

sensitive to changes in steeping and germination procedure; that diastatic power is very sensitive to changes in kilning procedure; and that malting loss and sprouts are very sensitive to changes in steeping and germination procedure but are not affected by changes in kilning procedure.

On the whole, the effects are small, but the fact that most of them are statistically significant shows that the equipment and malting method are sufficiently precise for investigation of the effects of small changes in the malting process. It is also apparent that the data may be useful in detecting sources of error in a malting test. For instance, if diastatic power can be determined precisely and malting loss cannot, then the fault must be sought in the steeping and germination equipment and not in the kilns.

The investigation demonstrates quite clearly that if the malting test is to be used merely for obtaining an estimate of extract yield, then comparatively crude equipment and methods will serve satisfactorily. On the other hand, if good estimates of diastatic power and malting loss are desired, every precaution will have to be taken to see that the control of the conditions under which the malts are made is precise.

The design of the investigation permits a study of more complicated aspects of the effects of different treatments. The possibility exists that changes in germination or kilning procedure will have different effects depending upon the moisture content to which the barley is steeped, or that changes in kilning will have different effects depending upon the germination procedure used. These so-called interaction effects have been investigated by means of statistical analyses of the data. Only two of the 21 interactions studied (three interactions for each of seven determinations) proved to be significant. Increasing the temperature of the germination chamber from 53° to 54.5° F. increased malting loss by 1.2% when the barley was steeped for 60 hr. and by only 0.7% when it was steeped for 48 hr. Lower temperatures during kilning increased diastatic power by 15.5° L. when the germination chamber was operated at 54.5° F. and by 14.1° L. when the chamber was operated at 53° F.

The study of the interaction effects should be of considerable interest to those working with the laboratory malting test. If numerous, comparatively large, interaction effects existed, considerable difficulty might be anticipated in developing a precise malting test, because errors introduced by variations at one stage of the process would be magnified by variations at another stage of the process. Moreover, if large interaction effects existed, it would be more difficult to investigate the effects of changing the conditions of a single stage of the malting process, because the effects of such changes would be dependent upon the conditions maintained in the rest of the process. In these circumstances simultaneous investigations of the effects of changing several factors would be required, a replication of malting units would be needed, and investigations of a factorial design would have to be undertaken.

The investigation provides some grounds for believing that serious complications of this sort will not arise. Only the interaction of germination

procedure on steeping procedure, with respect to malting loss, is of sufficient magnitude to merit further consideration. The existence of this interaction effect, together with the fact that malting loss is very sensitive to changes in both steeping and germination procedure, suggest that it will be fairly difficult to develop a malting test which is precise with respect to the determination of malting loss.

Statistical Analyses

For each determination, the variance of the data was analyzed into portions due to: (i) variations in the general level of results obtained in different batches; (ii) average differences, over all batches, between treatments; (iii) differences in the relative performance of treatments in different batches; (iv) differences between duplicate malts, and (v) differences between duplicate analyses. The variance due to average differences, over all batches, between treatments, was then analyzed into portions due to (vi) average differences between steeping procedures; (vii) average differences between germination procedures; (viii) average differences between kilning procedures; (ix) the interaction between steeping and germination procedures; (x) the interaction between steeping and kilning procedures; (xi) the interaction between germination and kilning procedures, and (xii) the triple interaction between steeping, germination and kilning procedures. The results of the analyses of variance are summarized in Table VI.

TABLE VI
ANALYSES OF VARIANCE FOR ALL DATA

Variation due to	Degrees of freedom	Mean squares						
		Extract, %	Moisture, %	Color units	Diastatic power, °L	Perm. sol. nitrogen, %	Malting loss, %	Sprouts, %
Treatments	7	.0424**	.0591**	.1139**	567.597**	.00824**	4.3089**	1.0771**
Batches	3	.0782**	.0217	.0203	216.710**	.01497**	2.6004**	.9247**
Treatments × batches	21	.0097	.0088††	.0101††	13.856††	.00048†	.1430††	.0207††
Steeping	1	.1008**	.0264	.0100	165.766**	.02911**	15.4056**	3.0976**
Germination	1	.0324	.0264	.0355	284.766**	.02743**	13.6900**	4.3681**
Kilning	1	.0930**	.3452**	.7225**	3507.601**	.00065	.1406	.0203
Steeping × germination	1	.0315	.0014	.0156	.090	.00033	.8557*	.0042
Steeping × kilning	1	.0204	.0001	.0039	1.434	.00001	.0626	.0086
Germination × kilning	1	.0183	.0126	.0025	8.492*	.00001	.0057	.0410
Steeping × germination × kilning	1	.0002	.0015	.0076	4.529	.00011	.0023	.0000
Duplicate malting tests	32	.0134	.0028	.0038	1.967	.00022	.0084	.0067
Duplicate analyses	64	.0098	.0029	.0048	1.277	.00053		

Double signs denote that the mean square attains a 1% level of significance, single signs denote a 5% level.

* and ** Significantly greater than the mean square due to treatments × batches.

† and †† Significantly greater than the mean square due to duplicate malting tests.

The significance of the results of the analyses was determined by application of the *Z* test and the standard errors, reported earlier in this paper, were calculated from the appropriate mean squares in the usual manner.

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AN INVESTIGATION OF STRAWBERRY VIRUS DISEASE IN ONTARIO¹

By R. V. HARRIS² AND A. A. HILDEBRAND³

Abstract

Following identification in 1932 of the Yellow-edge virus disease in England on the Royal Sovereign variety, "normal" plants of this variety from a clone minutely rogued for Yellow-edge were used at St. Catharines as indicators in a further study of virus as relating to certain Ontario varieties. Observations in the field and greenhouse, confined largely to the varieties Parson's Beauty, Premier (Howard 17), Forward and Glen Mary, showed that symptoms analogous to those of Yellow-edge in England and sufficiently defined to permit of diagnosis were apparent only on Parson's Beauty and Forward, and then only for a limited period early in the growing season. In the 1933-35 transmission experiments (by runner grafting), symptoms macroscopically indistinguishable from those of typical Yellow-edge-infected plants in England were induced on Royal Sovereign from the local varieties Glen Mary, Parson's Beauty and Premier, which possess markedly the symptomless-carrier capacity. Of special interest was the deterioration of Premier components in certain graft series, the evidence suggesting reciprocal infection between test and indicator plants.

Finally, parallel experiments at the East Malling Station in 1935-36 provided supplementary data as follows: (1) Of the two parent *Fragaria* species common to commercial varieties in North America and in England, *F. chiloensis* was found to be a symptomless-carrier of Yellow-edge with a high order of resistance, and *F. virginiana*, in complete contrast, exhibited symptoms with extreme readiness together with high susceptibility, thus providing some explanation of the observed wide range of varietal reaction to disease of the Yellow-edge type. (2) A large proportion of the clone of Royal Sovereign plants used as "normal" indicators in the recent series of experiments, was found to be infected with a distinct virus of the "Crinkle" type, thus providing explanation of an observed reciprocal reaction in certain series with the Premier variety.

General Introduction

As the result of investigations begun in 1931 at East Malling, the senior author determined the incidence of a virus disease of strawberries in commercial stocks of the Royal Sovereign variety in southwestern England (3). At that time the close similarity between the disease in England and Xanthosis or Yellows in California (9) was pointed out and the popular descriptive name of Yellow-edge was proposed for the former. Subsequently, a year's residence in eastern Canada, at the Dominion Laboratory of Plant Pathology, St. Catharines, Ontario, from May, 1933 (under an exchange arrangement with Dr. G. H. Berkeley, Senior Pathologist-in-charge of that laboratory) provided the senior author with valuable opportunity for extending his field of reference in the study of virus diseases of the strawberry.

A preliminary survey of experimental and commercial plantations in the Niagara Peninsula was followed up experimentally by attempts to infect "normal" plants of the English indicator variety, Royal Sovereign, (similar in clonal origin to those used in the original East Malling experiments and

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shipped to St. Catharines for the purpose) from plants of local varieties widely grown in Ontario, using the method of grafting (runner-inarching) devised at East Malling (2). Throughout these preliminary studies the senior author had the advantage of collaboration with the junior author, who was at that time commencing a study of a serious form of failure of the root-rot type, in local strawberry plantations (5). The initial small-scale experiments on virus disease yielded results indicating that the incidence of such disease on local varieties was more widespread, and of a more serious nature than was apparent from symptoms visible in the field; and the question arose as to the freedom from virus of the plant material of certain varieties used in the root-rot experiments. At the suggestion of the Dominion Botanist, Dr. H. T. Güssow, therefore, a scheme for future experiments, designed to test and extend the preliminary work of 1933, was drawn up jointly by the present writers, and suitable plant material selected and prepared before the termination of the exchange period. Thereafter the investigation at St. Catharines was carried on by the junior author whilst the senior author resumed his experiments at East Malling on the English-grown varieties, with particular reference to Royal Sovereign.

Early in 1936 an account of the experiments carried out at St. Catharines in 1934 and 1935 was forwarded to East Malling. The results of these were found in the main to confirm the conclusion tentatively reached in 1933 and further to indicate that virus disease in Ontario was more general in occurrence than had been realized.

At the same time complementary results were forthcoming from the further experiments with English-grown varieties at East Malling in 1935, certain of which indicated that the clonal race of Royal Sovereign plants used in the St. Catharines experiments and tentatively regarded as "virus-free" was actually infected with virus, distinct, however, from Yellow-edge virus. This conclusion was amply confirmed by a large-scale experiment in 1936 and provides an explanation of an otherwise anomalous and inexplicable reciprocity in the reaction of certain series of Premier-Royal Sovereign grafts in the 1935 St. Catharines experiments.

In the pages that follow, the share of the investigation borne by each of the writers has been indicated by placing the initials of the worker primarily responsible after the heading of each constituent section of the paper.

Initial Investigations at St. Catharines, 1933-34 (R.V.H.)

A. FIELD SURVEY

1. *Variety Parson's Beauty*

Early in June, 1933, extensive plantations of this variety at St. Davids, Ontario, were examined.* The growers reported a progressive falling-off of vigor and cropping in this stock of plants during the preceding seasons, culminating in the 1933 season when the bulk of the plants were found to be

* The writer accompanied Mr. G. C. Chamberlain, Acting Officer-in-charge of the St. Catharines laboratory, on this visit, and is grateful to Mr. Chamberlain for drawing his attention to the symptoms in question.

in an advanced stage of deterioration. At this time the majority exhibited leaf symptoms similar to those of Yellow-edge in Royal Sovereign in England, but in no case so clearly pronounced, particularly in the matter of marginal chlorosis.

In all subsequent visits by both writers to these plantations the symptoms were found to have become completely masked and diagnosis impossible. At the time of the first visit, infected plants showing the typical symptoms were selected and transferred to pots in the greenhouse for experimental purposes.

2. *Variety Premier (Howard 17)*

In June, 1933, Dr. J. H. L. Truscott (11) at the Vineland Horticultural Station drew the attention of the senior writer to a series of plants of this variety recently collected from a local deteriorating plantation. Dr. Truscott stated that attempts to isolate pathogenic fungi from the roots of these plants had given negative results, but that a proportion of the plants showed slight symptoms of the so-called "mosaic" (1) or June Yellows (10) disease. The plants were taken to St. Catharines for further observation and were used in the experiments to be described below. During the subsequent period of observation and experimentation, a proportion of the plants and their progeny showed symptoms of "mosaic" but at no time was any trace of symptoms analogous to those of Yellow-edge (Xanthosis) detected. When the writers visited the plantation of origin with Dr. Truscott in June, 1934, a proportion of the plants were found to show "mosaic" (June Yellows) and mite infestation symptoms, but again it was impossible to find symptoms of Yellow-edge.

This variety is extremely susceptible to root rot (5) and numerous visits were made to plantations affected by this disease. In view of results from the concurrent virus transmission experiments, a special look-out was kept for symptoms of the Yellow-edge type, but except for plants that were found to be "flat" in appearance, with very slightly chlorotic leaves, no sufficiently distinct and clear-cut symptoms were observed in this variety to make approximate field diagnosis or rogueing even remotely possible.

3. *Other Varieties*

During the 1933 season one of the writers (A.A.H.) carried out extensive experiments with the variety Glen Mary in connection with his root-rot investigations (5). Plants of this variety, in both commercial and experimental plantings, were kept under constant observation for symptoms of Yellow-edge, but no symptoms similar to those previously seen on Parson's Beauty at St. Davids were observed.

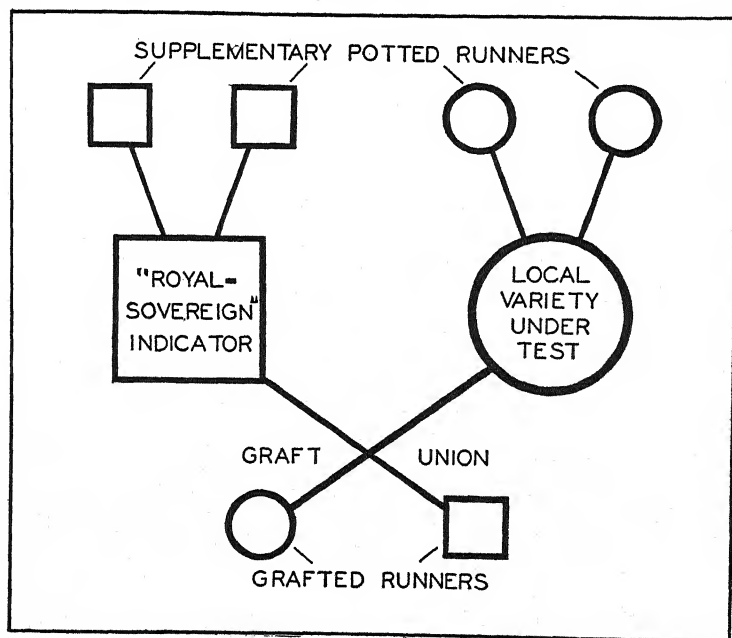
In a small greenhouse population of the variety Forward observed by the senior author for some months after his arrival at St. Catharines, one plant showed symptoms closely analogous to those of Yellow-edge. No runners were produced by this plant or others of the group so that it was not possible to carry out grafts on to Royal Sovereign as indicator.

During visits to the Central Experimental Farm at Ottawa, in June and September, 1933, and July, 1934, surveys were made of extensive collections of seedlings raised by M. B. Davis of the Division of Horticulture. The majority of these were free from distinguishable symptoms of Yellow-edge, but certain of them showed symptoms similar to those seen on Parson's Beauty and Forward.

1. Material and Methods

B. EXPERIMENTAL

All the "normal" Royal Sovereign plants (termed indicators below) used throughout the entire series of experiments at East Mallings and at St. Catharines, referred to in this communication, were derived from a clonal family originating from a single plant in 1928 and subsequently subjected continuously to direct measures for pest control and to regular inspections, re-selections, and rogueing to eliminate plants showing lack of health and vigor in general, and the Yellow-edge disease in particular. Of the 50 "normal" indicator plants in the first consignment shipped to St. Catharines in March, 1933, only seven plants survived, but these developed into uniformly vigorous and healthy plants and were ready for grafting early in August. The only impediment to their development was severe infestation with mite (*Tarsonemus pallidus* Bks.) which appeared early in June on all plants in this series, and also on the Forward, Parson's Beauty and Premier series. All plants were therefore subjected to two applications of the warm water treatment (in June and September, 1933, respectively) to control this pest (6).



TEXT-FIG. 1. Plan of graft-unit

The method of grafting used was that of runner-inarching as in the East Malling experiments (2) with an improved binding medium substituted for the old raffia and wax, namely, a proprietary sheet form of self-sealing, pure crêpe rubber.* With this material a graft union can be thoroughly sealed and bound rigidly and permanently in place in a single operation.

The general scheme of a graft-unit is shown in Text-fig. 1. In the East Malling 1932 experiments the passage of the virus from infected to healthy plants via the inarched stolons, and thence to all the runner progeny, was found to be extremely rapid and it was, therefore, decided in the present case not to strike into pots the runners on the grafted stolons, but to pot instead at least two ungrafted (supplementary) runners each from the Royal Sovereign indicator plants and from the plants of the local variety under test.

2. *Experiments with Parson's Beauty*

Of the eight plants showing symptoms analogous to Yellow-edge, collected from St. Davids, only two eventually produced runners suitable for grafting. Two graft-units were set up with these on August 5, each of which made union.

On September 19, at the time of the second warm water treatment, the familiar symptoms of the Yellow-edge type were visible on both Royal Sovereign parent indicator plants in spite of the masking effect of mite, which had completely obscured Yellow-edge symptoms on Parson's Beauty.

On October 30, distinct Yellow-edge symptoms were recorded on all components of the indicator variety in both units (Plate I, Fig. 1). Of the Parson's Beauty plants, both grafted runners showed clear symptoms, one of the parent plants showed symptoms very indistinctly, the other not at all, and no symptoms were recorded on any of the supplementary runners. Thereafter, the symptoms became completely masked on the Parson's Beauty plants but persisted on the indicators. The latter rapidly deteriorated in vigor of growth until March, 1934, when the stunting had become extreme (Plate II, Fig. 1). Ungrafted indicator checks which received the same treatment for mite remained free from Yellow-edge symptoms and developed normally and vigorously throughout (Plate II, Figs. 1 and 2).

3. *Experiments with Premier*

No symptoms analogous to Yellow-edge were detected at any time on the six plants of this variety obtained from Vineland. Three of them showed the distinct and characteristic symptoms of "mosaic" (June Yellows) transitorily and slightly at the time of transfer to St. Catharines.

Only two plants produced runners suitable for grafting; one of these belonged to the "mosaic" group. Two units were set up with these plants on August 5, 1933, and in both cases union took place.

At the time of the second warm water treatment on September 19, no definite virus symptoms were visible on any of the indicator plants and runners. On October 30, definite symptoms, closely resembling those of Yellow-edge, were recorded on the parent indicator plant and on one of

* Supplied by the Sterling Rubber Company, Guelph, Ont., under the name of Sterlaid.

the supplementary indicator runners of one of the units (Plate I, Fig. 2). Later, (unlike the indicators in the Parson's Beauty experiment) the symptoms on these plants became masked and it was not until March, 1934 that they reappeared distinctly. At this time also, and not till then, definite Yellow-edge symptoms were recorded on the parent plant and on supplementary runners of the remaining unit. At no time during the experiment were symptoms of the Yellow-edge (Xanthosis) type recorded on the test (Premier) plants or their runners (Plate II, Fig. 2). Finally, the rate of deterioration of the indicators in the Premier experiment was not so rapid as in the Parson's Beauty experiment (Plate II, Fig. 1).

As has already been recorded in the account of the former experiment, the ungrafted indicator checks and their progeny remained normal and vigorous throughout.

C. DISCUSSION OF RESULTS AND CONCLUSIONS

Of the local varieties studied, symptoms analogous to Yellow-edge on Royal Sovereign were observed only on Parson's Beauty (in the field and in the greenhouse) and on a single plant of Forward (in the greenhouse). These symptoms were at no time as pronounced as those on Royal Sovereign plants in plantations in England or infected from Parson's Beauty in the greenhouse at St. Catharines. Further, the tendency for the diagnostic symptoms to become masked (symptomless-carrier capacity) was found to be much more pronounced in the two local varieties than in Royal Sovereign plants infected from these varieties and kept under identical (greenhouse) conditions. Further, rapid symptom recession or masking took place in the field following the initial record of symptoms on Parson's Beauty in June, 1933, and persisted throughout the remainder of the senior writer's residence in Canada. A similar progressive symptom-masking (particularly in the matter of the marginal leaf chlorosis) was also recorded on the plants of both the above local varieties in the greenhouse, until in October no definite symptoms could be detected, although such were distinctly manifest at the same time on the parallel Royal Sovereign indicator plants.

That the Premier variety possesses the symptomless-carrier capacity to an even more marked degree than Parson's Beauty is suggested by the fact that, although at no time during the investigations were any diagnostic symptoms recorded in any field plantation of this variety or on any single plant in the greenhouse, certain of the latter plants were shown to be infected with virus (of the Xanthosis-Yellow-edge type) by the use of Royal Sovereign plants as indicators.

Thus, it would appear that a survey of the incidence of virus disease on local varieties in Ontario based on macro-symptoms in the field, bears but little relation to the distribution of infected plants.

Although, as noted above, under uniform greenhouse conditions a distinct contrast was recorded (both in degree of clearness and in tendency to masking) between the symptoms shown by Parson's Beauty and Forward, and those

induced in the parallel Royal Sovereign indicator plants (infected from both Parson's Beauty and Premier), on the other hand, the symptoms appearing on the indicators were indistinguishable from those of Yellow-edge occurring naturally or induced artificially on that variety in England. This implies that any difference between the symptomatological pictures in both countries is related primarily to differences inherent in the varieties grown, rather than to wide differences between the pathogens or the environmental conditions of cultivation. Thus the close similarity between Yellow-edge disease in England and the analogous disease in Ontario is emphasized.

Finally, the lack of vigor and the markedly poor performance of the infected plants of both Premier and Parson's Beauty suggest that although these varieties, and particularly the former, possess a capacity for acting as symptomless-carriers (for the Xanthosis-Yellow-edge type of disease) to a degree greatly in advance of Royal Sovereign, they are both also susceptible to the deteriorating action of the disease. That their degree of susceptibility is, however, lower than that of Royal Sovereign is indicated by the relatively rapid deterioration of plants of the latter variety following infection from the former by grafting.

Further Experiments at St. Catharines 1934-35 (A.A.H.)

MATERIAL AND METHODS

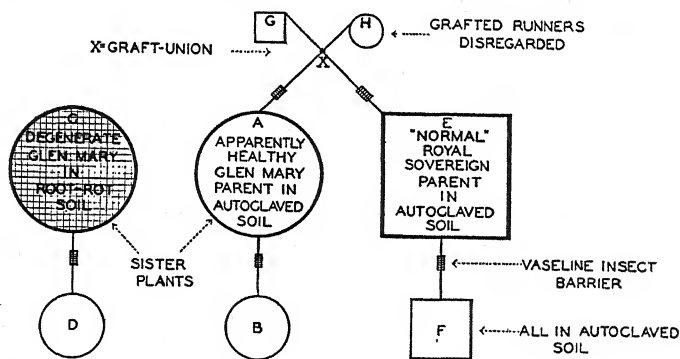
In the experiments carried out by the junior author, attempts at transmission of virus were limited to grafting, the technique of runner-inarching being the same as that already employed by the senior author. In outdoor experiments, however, it was found necessary to supplement the crêpe rubber sealing and binding newly grafted stolons, with an outer wrapping of raffia, to prevent too rapid deterioration of the rubber in direct sunlight.

Attention was concentrated on three local varieties only, namely, Glen Mary, Parson's Beauty and Premier. Stolons from selected specimens of these varieties were grafted to those of indicator plants, variety Royal Sovereign, the clonal origin of which was similar to that of plants used in the same capacity by the senior author in his experiments at East Malling and at St. Catharines. These plants were regarded at the time as virus-free, but as intimated in the general introduction, research in England subsequent to the experiments carried out at St. Catharines showed that this clonal race of Royal Sovereign plants was actually infected with virus, distinct, however, from Yellow-edge. This accounts for repeated reference to these as "normal" rather than as virus-free plants.

In all, 10 different series of grafts involving 61 individual graft-units were completed, some in the greenhouse, others in outdoor plots. Where possible, all component plants of a series were grown in autoclaved soil, thus eliminating the effect of soil organisms as a complicating factor. Facilities were not available for the prevention of infestation of plants by insect pests but, when necessary, remedial measures were adopted, as, for example, periodic warm water treatments for the control of the Tarsonemid mite (6).

*Series I**Variety: Glen Mary*

In 1933, as reported elsewhere (5), significant differences in the vigor of growth of runners were obtained by training each member of a series of clonal pairs of runners of the variety Glen Mary, (i) in autoclaved greenhouse compost and (ii) in non-sterilized soil from a plantation seriously affected with root rot. The resultant vigor of growth of the plants in the former was significantly greater, both as regards aerial parts and root systems. These observed differences were found to be correlated with the comparative freedom from fungi and nematodes of the roots of runners grown in the sterilized soil and with an abundance of these organisms in the roots of those grown in the non-sterilized plantation soil. It was decided that it might be interesting to carry the experiment further and test the sets of genetically identical runner plants for the presence or absence of virus. Consequently, on June 8, 1934, the stolons of five apparently healthy Glen Mary plants raised in autoclaved soil in the greenhouse were grafted to those of "normal" Royal Sovereign plants, and on July 8, five similar grafts were made. As they became available, runners from each of the parents were struck in autoclaved soil. Also, as they became available, runners produced by the "degenerate" Glen Mary component of each clonal pair were struck in autoclaved soil. The scheme of grafting and disposition of individual plants comprising a typical graft-unit of Series I is illustrated graphically in Text-fig. 2.



TEXT-FIG. 2. Scheme of grafting and disposition of individual plants comprising a typical, fully completed Glen Mary X Royal Sovereign graft-unit (Series I).

The plants involved in the experiment were kept in the greenhouse for almost a year. To afford them full opportunity for development, they were transferred to new soil (autoclaved) in larger pots during the winter, and to preclude the masking effect of mite injury, they were periodically given the warm water treatment. The results appearing in Table I represent the final decisions arrived at, following a series of observations which finally terminated on June 5, 1935, after the plants had passed through the spring period of renewed growth activity.

By July 24, that is, within seven weeks from the time the grafts had been made, indicator plants exhibited typical symptoms of Yellow-edge as evidenced

by (i) chlorosis or yellowing of the marginal region of the leaflets; (ii) an abnormally "flat" appearance, the foliage consisting of a zone of more or less normal outer leaves enclosing a central zone of dwarf or Yellow-edge leaves (Plate III, Fig. 1, E and F). By October the general symptomatological picture had changed, the diagnostic symptoms now chiefly in evidence being a general dwarfing of the plant, an irregular curling of the marginal regions of the leaflets, abnormally short petioles and a more or less general distortion and asymmetrical appearance of the leaves (Plate III, Fig. 2, E and F). In this experiment, once a Royal Sovereign plant became infected, it never subsequently showed other than very limited power of recovery and none of the affected plants produced stolons.

During the last week in July, when the Royal Sovereign runner plants of the graft-units were unmistakably showing symptoms of Yellow-edge, the soil was washed from their roots, which were then compared with the roots of "normal" non-grafted, Royal Sovereign runner plants of approximately the same age (about nine weeks). Macroscopically it was at once apparent that the roots of the Yellow-edge plants lacked the general bulk of those of the healthy, non-grafted plants. Microscopical examination showed that, though the root systems of both grafted and non-grafted plants were not free from organisms—fungi and nematodes,—such organisms were present at this time in such relatively small numbers that they could not be held accountable for the dwarfed condition of the roots of the plants involved in the graft-units.

Complete results as summarized in Table I show (i) that all graft-unions were successful, (ii) that seven of the ten degenerate Glen Mary plants raised in the non-sterilized plantation soil produced runners which when struck in autoclaved soil developed into vigorous, healthy plants, (iii) that all the Glen Mary plants and their runner progeny remained healthy except for insect injury in some cases, and (iv) that all the Royal Sovereign plants and the runner progeny of each developed symptoms indistinguishable from those of Yellow-edge. Before the termination of the experiment, four of the affected Royal Sovereign plants had died (Units 5, 6, 8 and 10), one was dying and the remainder were showing varying stages of deterioration. Like the Glen Mary plants, the Royal Sovereign plants no doubt also suffered injury from insect attack, but, in their more or less degenerate condition, the latter was difficult to distinguish as such and certainly was not of sufficient consequence materially to affect correct interpretation of the results.

Series II

While the results obtained in the greenhouse experiments in 1934 seemed sufficiently conclusive, it nevertheless appeared advisable to reserve final decision until a graft series could be completed under outdoor conditions. Consequently, on July 3, 1935, eight Glen Mary plants, chosen because of their exceptionally healthy and vigorous appearance from a population of this variety growing in outdoor plots, were runner-grafted to "normal" Royal

TABLE I
RESULTS OBTAINED FOLLOWING GRAFTING OF STOLONS OF APPARENTLY HEALTHY GLEN MARY PLANTS WITH THOSE OF "NORMAL"
ROYAL SOVEREIGN PLANTS

Graft series	Graft-unit No.	Date of graft	Location of experiment	Result of graft-union	Condition of plants subsequent to grafting					
					Glen Mary			Royal Sovereign		
					D	B	A	E	F	
					Runner from degenerate parent	Runner	Parent	Parent	Runner	
I	1	8.6.34	Greenhouse	+	Healthy	Healthy	Healthy M*	Y.E.*	Y.E.	
I	2	8.6.34	Greenhouse	+	Healthy	Healthy	Healthy	Y.E.	Dying	
I	3	8.6.34	Greenhouse	+	Healthy	Healthy	Healthy S*	Y.E.	Y.E.	
I	4	8.6.34	Greenhouse	+		Healthy	Healthy	Y.E.	Y.E.	
I	5	8.6.34	Greenhouse	+	Healthy	Healthy	Healthy	Y.E.	Dead	
I	6	8.7.34	Greenhouse	+	Healthy	Healthy S	Healthy MS	Dead	Y.E.	
I	7	8.7.34	Greenhouse	+	Healthy	Healthy	Healthy	Y.E.	Y.E.	
I	8	8.7.34	Greenhouse	+		Healthy M	Healthy	Y.E.	Dead	
I	9	8.7.34	Greenhouse	+		Healthy	Healthy	Y.E.	Y.E.	
I	10	8.7.34	Greenhouse	+	Healthy	Healthy	Healthy	Dead	Y.E.	

TABLE I—*Concluded*
 RESULTS OBTAINED FOLLOWING GRAFTING OF STOOLONS OF APPARENTLY HEALTHY GLEN MARY PLANTS WITH THOSE OF "NORMAL"
 ROYAL SOVEREIGN PLANTS—*Concluded*

Graft series	Graft-unit No.	Date of graft	Location of experiment	Result of graft-union	Condition of plants subsequent to grafting					
					Glen Mary			Royal Sovereign		
					D	B	A	E	F	
					Runner from degenerate parent	Runner	Parent	Parent	Runner	
II	11	3.7.35	Outdoors	+		Healthy	Healthy	Y.E.	Y.E.	
II	12	3.7.35	Outdoors	—		Healthy	Healthy	Healthy	Healthy	
II	13	3.7.35	Outdoors	+		Healthy	Healthy	Healthy	Healthy	
II	14	3.7.35	Outdoors	+		Healthy	Healthy	Healthy	Healthy	
II	15	3.7.35	Outdoors	+		Healthy	Healthy	Y.E.	Y.E.	
II	16	3.7.35	Outdoors	—		Healthy	Healthy	Healthy	Healthy	
II	17	3.7.35	Outdoors	+		Healthy	Healthy	Y.E.	Y.E.	
II	18	3.7.35	Outdoors	+		Healthy	Healthy	Y.E.	Y.E.	

* M = *mile injury*; Y.E. = "*Yellow-edge*"; S = *red spider injury*.

Sovereign plants, transferred from the greenhouse in the pots in which they had been raised. As checks, four additional "normal" Royal Sovereign plants were transferred to the outdoor environment. A runner from each parent in a graft-unit was struck in autoclaved soil. Eight graft-units comprised the series.

The Royal Sovereign plants, upon being removed to the outdoor plots, proved to be very susceptible to attack by the leaf-spot organism, *Mycosphaella fragariae* (Schw.) Lind., the infection becoming so severe as to render it extremely difficult to evaluate the possible effect of any other disease-producing agent. Critical diagnosis of symptoms apart from leaf-spot was therefore not attempted under outdoor conditions. On September 19, all Royal Sovereign plants, both parents and runner progeny, also the runner progeny of the Glen Mary parents, were transferred back to the greenhouse. All leaves affected with leaf-spot were clipped off, the plants were given the warm-water treatment to rid them of possible mite infection and then they were transferred to new soil (autoclaved) in larger pots. The plants then developed relatively free from leaf-spot and mites, and in a series of observations it was possible to evaluate the effect due presumably to grafting alone.

As reference to Table I will show, all but two of the graft-unions were successful. It seems significant that in the two cases of failure (Units 12 and 16) the indicator Royal Sovereign plants remained healthy. Of significance also, is the fact that in two cases where the grafts *were* successful, symptoms of Yellow-edge did not appear in the Royal Sovereign components (Units 13 and 14). In the remaining four cases symptoms characteristic of Yellow-edge developed in the Royal Sovereign plants (Units 11, 15, 17 and 18). The four check plants, though attacked outdoors by leaf-spot and possibly by mites, recovered later in the greenhouse and did not show symptoms of Yellow-edge.

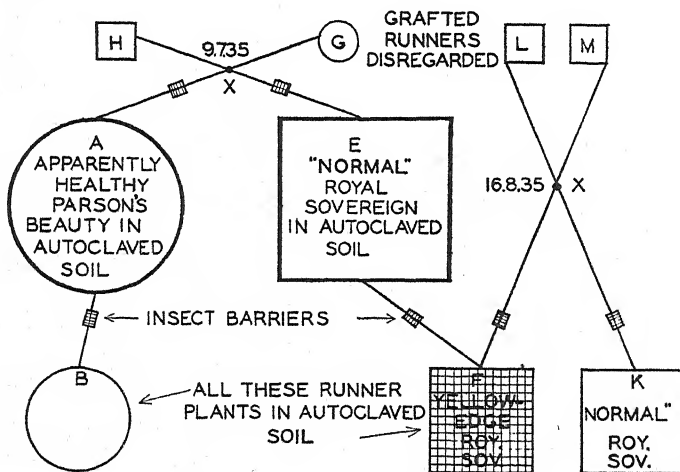
Discussion Regarding the Variety Glen Mary

Following successful grafting of the stolons of Glen Mary plants selected because of their apparent health and vigor, to those of "normal" Royal Sovereign plants, 14 parent plants (out of a possible 16) of the latter variety, and runner progeny from each, developed symptoms indistinguishable from those of Yellow-edge as described on the English-grown variety. This may be taken as almost conclusive evidence that plants of the Ontario-grown variety are symptomless carriers of the disease. That this capacity is associated with a high degree of resistance to this particular disease is shown by the fact that Glen Mary plants from which the disease was transmitted to the indicator variety, continued to manifest the outstanding vigor of growth that is characteristic of the variety, both in the greenhouse and in an outdoor environment. Since, following two successful graft-unions, the indicator Royal Sovereign plants remained unaffected, the inference would be that some Glen Mary plants have not become infected with (the) virus.

*Series III**Variety: Parson's Beauty*

In the fall of 1933 the present investigators inspected the plantation at St. Davids where, earlier in the season, the senior author had obtained suspect plants, variety Parson's Beauty, from which he was successful in transmitting Yellow-edge to "normal" Royal Sovereign plants (4). On the occasion of the second inspection, no plants could be found that exhibited characteristic symptoms of Yellow-edge, other than some that presented an abnormally "flat" appearance. A number of these were transferred to the greenhouse and in the spring of 1934 runners produced by them were struck in autoclaved soil. These runners developed into plants apparently healthy and true to variety. They were kept under observation until July 9, on which date seven of them were runner-grafted with "normal" Royal Sovereign plants.

In each of three graft-units of this series a runner-plant from the grafted Royal Sovereign (indicator) parent was itself runner-inarched to a further ("independent") "normal" Royal Sovereign indicator. The scheme of grafting of the three fully-completed units of the series is illustrated graphically in Text-fig. 3.



TEXT-FIG. 3. Scheme of grafting and disposition of individual plants comprising a typical fully-completed Parson's Beauty X Royal Sovereign graft-unit (Series III).

Reference to Table II will show (i) that all graft-unions were successful, (ii) that all but two of the Parson's Beauty plants, parents and runner progeny, remained healthy except for injury by insects in certain cases, and for apparent lack of vigor in two plants (Units 21 and 23), which, however, displayed no symptoms suggestive of any specific disease of strawberry known to the writer, (iii) that all the Royal Sovereign parent plants with one doubtful exception (Unit 22), and the progeny of the five that *did* produce runners, developed symptoms indicative of Yellow-edge and (iv) that the three Royal Sovereign runner plants to which Yellow-edge had been transmitted through

TABLE II

RESULTS OBTAINED FOLLOWING GRAFTING OF STOLONS OF APPARENTLY HEALTHY PARSON'S BEAUTY PLANTS WITH THOSE OF "NORMAL" ROYAL SOVEREIGN PLANTS

Graft-series	Graft-unit No.	Date of graft	Location of experiment	Result of graft-union	Condition of plants subsequent to grafting				
					Parson's Beauty		Royal Sovereign		
					B	A	E	F	K
					Runner	Parent	Parent	Runner	Independent
III	19	9.8.34	Greenhouse	+		Healthy	Y.E.*		
III	20	9.8.34	Greenhouse	+	Healthy	Healthy S*	Y.E.	Y.E.	Y.E.
III	21	9.8.34	Greenhouse	+	Healthy	Abnormal	Y.E.		
III	22	9.8.34	Greenhouse	+	Healthy	Healthy	Doubtful	Y.E.	Y.E.
III	23	9.8.34	Greenhouse	+	Abnormal	Healthy M*	Y.E.	Y.E.	Y.E.
III	24	9.8.34	Greenhouse	+	Healthy	Healthy M	Y.E.	Y.E.	
III	25	9.8.34	Greenhouse	+	Healthy	Healthy	Y.E.	Y.E.	

* Y.E. = "Yellow-edge"; S M = injury by red spider and mite, respectively.

their respective parents from the apparently healthy Parson's Beauty plants, in turn, transmitted the disease when grafted back to the three independent "normal" Royal Sovereign plants (Units 20, 22 and 23).

Discussion Regarding the Variety Parson's Beauty

Since "normal" Royal Sovereign plants, subsequent to being runner-grafted to apparently healthy Parson's Beauty plants, developed typical symptoms of Yellow-edge, it is evident that plants of the latter variety, like those of the variety Glen Mary *can* be symptomless-carriers of (the) virus of Yellow-edge. That symptoms of the disease may, however, be manifest in plants of the variety is indicated by Harris' original discovery of Yellow-edge in Parson's Beauty plants in Ontario (4).

Series IV

Variety: Premier

Because of the interesting and significant results that had already been obtained by the senior author in his experiments involving plants of the Premier variety, it was deemed advisable at the time to extend the investigations to include a larger number of plants of this widely grown variety. In the fall of 1933, the present investigators inspected a plantation of Premier plants where, during the summer, root rot had been severe. Thirty plants were brought back to the laboratory, 20 being chosen as apparently healthy, the other ten being selected because of their subnormal vigor and somewhat "flat" appearance, the latter condition being considered as a "flat" condition.

of virus infection. These plants were potted in fertile soil, and during the ensuing winter they received special attention. After the period of renewed growth activity in the spring, it became evident that only a few of the plants could be regarded as "normal". The majority of them developed an abnormally large number of leaves, smaller than those of "normal" plants, which were supported on long, slender petioles. In general, they bore considerable resemblance to Zeller's illustration of plants affected with Witches'-broom (12, p. 332, Fig. 3). Eventually, only three of the original 30 plants produced runners suitable for grafting, and curiously enough, these three plants were a part of the number that had been originally chosen as suspects. One of the three could be regarded as a "normal" Premier plant, while the other two exhibited slight traces of the abnormalities described above. At no time did these or any of the remaining 27 plants exhibit symptoms at all suggestive of Yellow-edge. These three plants were runner-grafted to "normal" Royal Sovereign plants on August 13, 1934, the three units comprising Series IV.

Referring to Table III, it will be noted that all Premier plants involved in Series IV, three parents and two runners, are recorded as healthy. Two of the parents (Units 26 and 27) were those which, before the grafts were made, had shown the Witches'-broom effect mentioned above. Since these two plants remained in very much the same condition subsequent to grafting and showed no symptoms at all suggestive of Yellow-edge, they are recorded as healthy. One of these plants, when runner-grafted to Royal Sovereign, produced no effect on the latter (Unit 26); the other, however, apparently had some effect on the Royal Sovereign component of the graft-unit, though the interpretation of the symptoms was doubtful. The runner in this unit developed symptoms of Yellow-edge. The results in connection with Unit 28 are interesting. This Premier parent was really the only "normal" vigorous plant of the series, yet within ten weeks after the grafts were made, both the Royal Sovereign parent and its runner showed typical and conspicuous symptoms of Yellow-edge, while the Premier and its runner were still in the state of health and vigor shown in Plate IV, Fig. 1.

The above small-scale experiments indicated that (i) the Witches'-broom effect, whatever its cause or nature, was not transmitted from Premier to Royal Sovereign; (ii) all Premier plants are not infected with (the) virus of Yellow-edge, and (iii) an apparently healthy Premier plant may be a symptomless-carrier of (the) virus of Yellow-edge.

Series V

When, early in 1934, it became apparent that the 30 plants mentioned above in connection with Series IV were not going to provide sufficient material for further grafting, runners being produced by Premier plants in an outdoor plot at the laboratory were struck in autoclaved soil in pots, June 3, 1934. Three weeks later, June 24, the young runner plants were transferred to the greenhouse, where they were kept under close observation for a month. During this period they made vigorous growth and produced robust stolons. On July 25, the stolons of seven of these Premier plants which

could not be considered as other than healthy, vigorous plants, were grafted to those of "normal" Royal Sovereign plants. Included in Table III are the summarized results of a series of observations extending from August, 1934, until after the period of renewed growth activity in the spring of 1935.

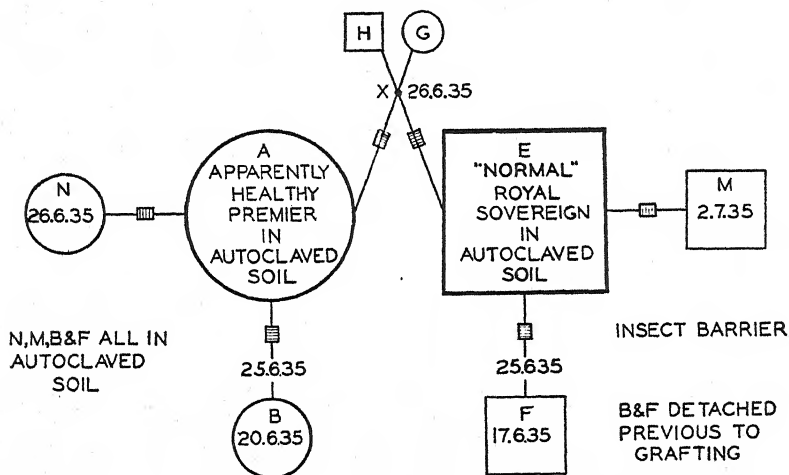
The results obtained in Series V were entirely different from those observed in any of the other series up to that point. Whereas in other experiments Royal Sovereign components alone had shown adverse effects subsequent to grafting, in this case Premier plants showed even more striking effects than did Royal Sovereign. Within a few weeks after grafting, symptoms of abnormality were already in evidence in certain of the Premier plants. The larger leaves began to die, the outer, older ones first, and then successively those formed later. Numerous new leaves were formed but these were small and were supported on short, variously twisted and distorted petioles, the combined effect giving a plant a dwarfed, "bunchy" appearance. At stages in the decline of the Premier plants the foliage showed discoloration and chlorosis but the chlorosis was not localized in the marginal regions of the undersized leaves. Since there was apparent in the Premier plants a gradation in severity of effect, a disease rating was assigned to each plant as shown in Table III, one plus sign indicating an almost healthy plant, while five denote a dead plant. It will be noted that two of the Premier plants died (Units 32 and 35). These plants having been raised in autoclaved soil, their death is all the more surprising since mortality of plants in the greenhouse even in root-rot soil has been exceptional in the experience of the writer. Microscopic examination of the roots of the Premier runner plants, made soon after the symptoms of abnormality had become apparent in the above-ground parts, showed that micro-organisms were not present in sufficient numbers to be held accountable for the condition of the plants. Further, whereas the roots of these Premier runners of the graft-units had ramified relatively sparsely through the soil, those of non-grafted Premier runners of approximately the same age, also grown in autoclaved soil, had formed solid, compact masses of intertwining roots to such an extent that the plants had become "pot-bound".

All the Royal Sovereign plants, except those in Unit 29, developed symptoms typical of those of plants affected with Yellow-edge. In the exceptional case the plants showed an abnormal condition, but the symptoms were not definitely those of Yellow-edge. The other Royal Sovereign plants, like the Premier components, showed differences in severity of effect and even within a given unit there were not necessarily correspondingly severe manifestations of disease on the part of all components (Units 32 and 35).

Series VI

In view of the unusual results obtained in Series V in 1934, it was decided to repeat the experiment. Consequently, as early as possible in June 1935, runners being produced outdoors by the Premier plants which had furnished the plants for Series V, were struck in autoclaved soil in pots. These were later transferred to the greenhouse, and after developing into robust plants as

their predecessors in 1934 had done, were runner-grafted to "normal" Royal Sovereign plants. As far as possible, parent plants which were producing at least three stolons were chosen for the series. Previous to grafting, one stolon from each parent was struck in autoclaved soil. Following detachment of these runner plants from their respective parents, the grafts were made using a second stolon from each plant, then subsequent to grafting, a third runner from each parent was struck in autoclaved soil. The date of grafting, the ages of the various components relative to this date, and the general disposition of the individual plants comprising a graft-unit of Series VI are shown in Text-fig. 4. Six graft-units were involved in the series and the results as finally interpreted are included in Table III.



TEXT-FIG. 4. Scheme of grafting and disposition of individual plants comprising a typical, fully completed Premier X Royal Sovereign graft-unit (Series VI).

All six grafts were successful. Before the termination of the experiment, the Premier parent plants became almost uniformly degenerate plants. In marked contrast with these, the runners detached from the parents previous to grafting developed into healthy plants, thus proving that the conditions which obtained in the greenhouse throughout the experiment were not of themselves detrimental to the growth of plants of this variety. The runners struck in autoclaved soil subsequent to the grafts being made, and left attached to the parent plants, consistently developed a condition intermediate between that of the healthy detached runners and the degenerate parent plants (Plate IV, Figs. 2 and 3). In Table III, their condition is recorded as abnormal with a two-plus disease rating.

All six Royal Sovereign parents developed symptoms of Yellow-edge as did also the plants developed from runners left attached after the grafts had been made. In this series, as in Series V, a gradation in severity of effect was noted and the plants were assigned a disease rating as shown in Table III. Of the six runners struck in autoclaved soil and detached from the parent

TABLE III

RESULTS OBTAINED IN GREENHOUSE EXPERIMENTS FOLLOWING GRAFTING OF STOLONS OF APPARENTLY HEALTHY PREMIER PLANTS WITH THOSE OF "NORMAL" ROYAL SOVEREIGN PLANTS

Graft series	Graft unit	Date of graft	Result of graft union	Premier				Condition of plants subsequent to grafting				Royal Sovereign			
				B		B		E		F		M			
				Runners left attached to parents	Disease rating	Parents	Disease rating	Parents	Disease rating	Runners left attached to parents	Disease rating	Runners detached previous to grafting	Disease rating		
IV	26	13.9.34	+			Healthy		Healthy		Healthy					
IV	27	13.9.34	+	Healthy		Healthy		Doubtful of interpretation		Y.E.					
IV	28*	13.9.34	+	Healthy		Healthy		Y.E.		Y.E.					
V	29	25.8.34	+	Abnormal	+++	Degenerate	+++	Doubtful of interpretation		Doubtful of interpretation					
V	30	25.8.34	+	Degenerate	+++	Degenerate	+++	Y.E.	+++	Y.E.	+++		+++		
V	31	25.8.34	+	Degenerate	++	Degenerate	++	Y.E.	+++	Y.E.	+++		+++		
V	32	25.8.34	+	Dead	++++	Almost dead	++++	Y.E.	++	Y.E.	++		++		
V	33	25.8.34	+	Degenerate	++	Degenerate	++	Y.E.	+	Y.E.	+++		+++		
V	34	25.8.34	+	Fairly healthy	+	Fairly healthy	+	Y.E.	++	Y.E.	++		++		
V	35	25.8.34	+	Dead	++++	Almost dead	++++	Y.E.	++	Y.E.	++		++		

* This unit photographed. See Plate IV, Fig. 1.

TABLE III—*Concluded*

RESULTS OBTAINED IN GREENHOUSE EXPERIMENTS FOLLOWING GRAFTING OF STOLONS OF APPARENTLY HEALTHY PREMIER PLANTS WITH THOSE OF "NORMAL" ROYAL SOVEREIGN PLANTS—*Concluded*

Graft series	Graft unit	Date of graft	Result of graft union	Condition of plants subsequent to grafting									
				Premier				Royal Sovereign					
				B		B		E		F		M	
				Runners detached previous to grafting	Runners left attached to parents	Disease rating	Parents	Parents	Disease rating	Runners left attached to parents	Disease rating	Runners detached previous to grafting	M
VI	36	26.6.35	+	Healthy	Abnormal	++							
VI	37	26.6.35	+	Healthy			Degenerate	Y.E.	+++	Y.E.	+	Y.E.	
VI	38	26.6.35	+	Healthy	Abnormal	++	Degenerate	Y.E.	++	Y.E.	+	Healthy	
VI	39	26.6.35	+	Healthy	Abnormal	++	Degenerate	Y.E.	+++			Healthy	
VI	40	26.6.35	+	Healthy			Degenerate	Y.E.	++	Y.E.	+++	Healthy	
VI	41	26.6.35	+	Healthy	Abnormal	++	Degenerate	Y.E.	++	Y.E.	+	Dead**	
							Degenerate	Y.E.	++			Healthy	

** Died as result of being detached too soon from parent.

plants previous to grafting, four remained healthy, one died (Unit 40, detached from the parent too soon), and one developed symptoms of Yellow-edge (Unit 36). The occurrence of the disease in the latter plant might be explained on the basis either of the parent plant already having become infected (though not showing symptoms) before the runner was detached, or of transmission of the virus from one of the numerous infected plants in the greenhouse, by an agent at present unknown.

Series VII-X

While the above-mentioned experiment was in progress in the greenhouse, another series of grafts was made under outdoor conditions. Early in the spring of 1935, Premier plants had been obtained from sources in Ontario as widely separated as Norfolk, Elgin, Wentworth and Lincoln Counties. These were planted in outdoor plots where they were kept under close observation. Later, during the period of prolific runner production, apparently healthy, vigorous plants were runner-grafted to "normal" Royal Sovereign plants, the latter being transferred from the greenhouse in the pots in which they had been raised. A runner from the parent of each variety was struck in autoclaved soil. Four series, VII to X inclusive, each series involving Premier plants from a different source and altogether comprising 20 graft-units, were completed within the period June 24-July 16, 1935.

As had been the case in a similar outdoor experiment (Series II above), the Royal Sovereign plants, soon after being removed to the outdoor environment, became so severely infected with strawberry leaf-spot that it was almost impossible to evaluate the possible effect of any other disease-producing agent. However, during the course of the summer certain of the leaves of the Royal Sovereign components of the graft-units showed most conspicuous marginal chlorosis. Early in September, all Royal Sovereign plants, also the runner progeny of the Premier plants, were transferred to the greenhouse where, after being treated as described for Series II, the plants developed relatively free from leaf-spot and injury by mites.

As reference to Table IV will show, only four of the grafts failed to make union. In two cases where the grafts were successful (Units 43 and 44), the Premier plants remained continuously healthy; the Royal Sovereign parents exhibited symptoms doubtful of interpretation but their runner progeny remained healthy. In two additional cases where the grafts had been successful (Units 56 and 61), the Premier components remained healthy while the Royal Sovereign plants developed symptoms of doubtful interpretation. In the remaining 12 cases of successful graft-unions, all the Premier plants and nine of their runner progeny remained healthy, whereas the 12 Royal Sovereign parents and the runner progeny of each developed symptoms indicative of the presence of Yellow-edge. The disease was transmitted from at least two Premier plants obtained from each of the four different localities. Judging from the severity of effect on the Royal Sovereign components, it would appear that the Premier plants obtained from Wentworth and Elgin Counties were more heavily infected with virus than those from Norfolk and

TABLE IV

RESULTS OBTAINED FOLLOWING GRAFTING OF STOLONS OF APPARENTLY HEALTHY PREMIER PLANTS OBTAINED FROM DIFFERENT SOURCES WITH THOSE OF "NORMAL" ROYAL SOVEREIGN PLANTS

Graft series	Graft unit	Date of graft	Result of graft union	Location of experiment	Source of Premier plants	Condition of plants subsequent to grafting					
						Premier		Royal Sovereign			
						B	A	E		F	
						Runner	Parent	Parent	Disease rating	Runner	Disease rating
VII	42	24.6.35	+	Outdoor plots	Norfolk County	Healthy	Healthy	Y.E.	++	Y.E.	+
VII	43	24.6.35	+	Outdoor plots	Norfolk County	Healthy	Healthy	Doubtful		Healthy	
VII	44	24.6.35	+	Outdoor plots	Norfolk County	Healthy	Healthy	Doubtful		Healthy	
VII	45	24.6.35	+	Outdoor plots	Norfolk County	Abnormal	Healthy	Y.E.	+++	Y.E.	+++
VII	46	3.7.35	-	Outdoor plots	Norfolk County	Healthy	Healthy	Healthy			
VII	47	3.7.35	-	Outdoor plots	Norfolk County	Healthy	Healthy	Healthy			
VIII	48	2.7.35	+	Outdoor plots	Lincoln	Healthy	Healthy	Y.E.	++	Y.E.	++
VIII	49	2.7.35	+	Outdoor plots	Lincoln	Healthy	Healthy	Y.E.	++	Y.E.	++
VIII	50	2.7.35	-	Outdoor plots	Lincoln	Healthy	Healthy	Healthy		Healthy	
VIII	51	2.7.35	-	Outdoor plots	Lincoln	Healthy	Healthy	Slightly abnormal		Healthy	
IX	52	3.7.35	+	Outdoor plots	Wentworth	Healthy	Healthy	Y.E.	+++	Y.E.	+
IX	53	3.7.35	+	Outdoor plots	Wentworth	Abnormal	Healthy	Y.E.	++	Y.E.	+++

TABLE IV—*Concluded*

RESULTS OBTAINED FOLLOWING GRAFTING OF STOLONS OF APPARENTLY HEALTHY PREMIER PLANTS OBTAINED FROM DIFFERENT SOURCES WITH THOSE OF "NORMAL" ROYAL SOVEREIGN PLANTS—*Concluded*

Graft series	Graft unit	Date of graft	Result of graft union	Location of experiment	Source of Premier plants	Condition of plants subsequent to grafting					
						Premier		Royal Sovereign			
						B	A	E		F	
						Runner	Parent	Parent	Disease rating	Runner	Disease rating
IX	54	3.7.35	+	Outdoor plots	Wentworth	Healthy	Healthy	Y.E.	+++	Y.E.	+
IX	55	3.7.35	+	Outdoor plots	Wentworth	Healthy	Healthy	Y.E.	++		
IX	56	3.7.35	+	Outdoor plots	Wentworth	Healthy	Healthy	Doubtful			
IX	57	3.7.35	+	Outdoor plots	Wentworth	Healthy	Healthy	Y.E.	++	Y.E.	++
X	58	⁹ 16.7.35	+	Outdoor plots	Elgin	Abnormal	Healthy	Y.E.	++	Y.E.	+
X	59	16.7.35	+	Outdoor plots	Elgin	Healthy	Healthy	Y.E.	+++	Y.E.	++
X	60	16.7.35	+	Outdoor plots	Elgin	Healthy	Healthy	Y.E.	+		
X	61	16.7.35	+	Outdoor plots	Elgin	Healthy	Healthy	Doubtful		Doubtful	

Lincoln Counties. In regard to the plants obtained from Norfolk County, it is worthy of mention that correlated with their apparent "milder" infection with virus was their outstanding vigor of growth.

Discussion Regarding the Variety Premier

Altogether, 36 Premier-Royal Sovereign runner-grafts were made, 16 in the greenhouse all successful, and 20 outside, four of the latter being unsuccessful. *It is significant that in all four cases of failure of graft-union (Units 46, 47, 50 and 51), the components of each variety remained healthy.*

In three cases of successful graft-unions (Units 26, 43 and 44), all Premier components remained healthy and none of the Royal Sovereign plants involved developed symptoms suggestive of Yellow-edge. The evidence in regard to the three present cases would suggest that the Premier parents were not infected with the virus of Yellow-edge and that inter-varietal "incompatibility" can be ruled out as a complicating factor in observed results.

In three other cases of successful graft-unions (Units 29, 56 and 61) the Royal Sovereign components all developed symptoms doubtful of interpretation, that is, the plants could not be regarded as normal; yet the symptomatological picture they presented could not be associated with any of the diseases of strawberry known to the writer. In one of the three cases under consideration (Unit 29, greenhouse), the Premier parent and its runner raised in autoclaved soil both became degenerate plants, but in the other two cases (Units 56 and 61, outdoors), the Premier parents and a runner from one of them remained healthy. The results obtained in these three cases suggest that virus other than that of Yellow-edge may be present in certain Premier plants.

In the remaining 26 cases of successful graft-unions, 12 outdoors and 14 in the greenhouse, symptoms of Yellow-edge developed in all the Royal Sovereign components. The results in regard to the Premier components are especially interesting. With two exceptions (Units 27 and 28), all Premier plants involved in greenhouse graft-units became more or less degenerate plants, as did also the runner progeny in Series V. In Series VI, the runners, which certainly were abnormal plants, did not approach the same degree of degeneracy. As sharply contrasted with results obtained in the greenhouse, all 12 Premier parents involved in the outdoor graft-units remained healthy, as did the runner progeny of nine of them, even after being transferred to the greenhouse in September. With regard to the anomalous decline of the Premier plants in Series V and VI the following explanations might be postulated. (i) The principal factor may be environmental, these experiments having been carried out in the greenhouse and the subsequent series (VII to X) in the open air. Against this it must be noted that the initial small-scale experiment in 1933 was carried out in the greenhouse and its results, as regards the behavior of the Premier plants, corresponded closely to those of the open air series (VII to X). Again, when the runner progeny of Premier parents, which in outdoor graft-series had transmitted Yellow-edge to Royal Sovereign plants, were transferred to the greenhouse they remained

in a healthy condition. Then, too, in Series VI, Premier runner plants detached from parent plants previous to grafting remained healthy, while at the same time, under identical conditions, sister plants left attached to the parents after the grafts had been made showed marked adverse effects while the parent plants themselves became degenerate plants. (ii) Up to this point the Royal Sovereign indicator plants have been assumed to be virus-free and therefore unlikely to have any reciprocal reaction on plants of the local test varieties runner-inarched to them. The anomalous behavior of the grafted Premier plants of the series in question would be comprehensible on the assumption that the indicator plants, although Yellow-edge-free, were infected with another pathogen (virus) and therefore had a reciprocal action on the test plants. That such was actually the condition of the indicator plants has been shown by the subsequent experiments with the Royal Sovereign clone at East Malling in 1935 and 1936, the results of which will be summarized in the following section of this paper.

Another point of interest as affecting Premier-Royal Sovereign grafts is the apparent gradation in severity of effect, especially in the series carried out in the greenhouse in which the effects appeared to be reciprocal. A glance at Table III will show that, as regards the Premier components, the range in effect is from fairly healthy (Unit 34) to dead plants (Units 32 and 35). This would suggest that all Premier plants are not infected to the same degree. Yet, if this were true, it would seem only logical to expect that there would be correspondingly slight or severe manifestations of disease on the part of both test and indicator plants of a given graft-unit. This is certainly not the case in Units 32, 35 and 40.

Both in outdoor and in greenhouse experiments all the Premier parent plants were kept under observation for some time before grafting, and then only those which gave every appearance of health and vigor were chosen. Yet in 26 cases, Royal Sovereign plants runner-grafted to these apparently healthy Premier plants developed the symptomatological picture associated with the Yellow-edge disease. Further, the disease was transmitted from at least two Premier plants obtained from each of four different localities in Ontario. It would appear, therefore, that not only are Premier plants symptomless-carriers of the virus of Yellow-edge, but that infection of plants of this variety is widespread in Ontario.

Further Experiments at East Malling, 1935-36* (R.V.H.)

In 1935 the scope of the experiments on the symptomatology of Yellow-edge was extended to include species of *Fragaria* and cultivated varieties additional to Royal Sovereign, using the latter variety both as a standard source of infection and as an indicator.

In these experiments plants of the varieties under test (test plants) were combined with Royal Sovereign in two types of graft-unit. (1) Test plants

* A detailed account of these experiments is in course of preparation for early publication. Here, the results are summarized in so far as they have a bearing on the results of the St. Catharines experiments.

were runner-inarched to "normal" Royal Sovereign indicators in order to determine possible infection in the absence of symptoms (symptomless-carriers). (2) Yellow-edge Royal Sovereign plants were runner-inarched to test plants and these again to "normal" indicators, in order to obtain comparative data on symptom expression in the variety under test. The results of these experiments to date, in so far as they have bearing on the present investigation, are briefly summarized below.

A. THE PARENT *Fragaria* SPECIES OF THE CULTIVATED STRAWBERRY

1. *Fragaria chiloensis* proved to be a symptomless-carrier of Yellow-edge. All of the large series of test plants used in this experiment were found to be Yellow-edge infected, and at no time has any trace of symptoms of this disease been recorded on any of the plants. The vigor of growth of all the plants (not markedly great at the beginning), has been uniformly maintained to the present time.

2. *Fragaria virginiana*, in complete contrast to the above, proved to be extremely susceptible to the disease in all respects. All the test plants were found to be free from infection at the start, but on being infected from Royal Sovereign they developed the complete range of Yellow-edge symptoms distinctly. These symptoms persisted continuously, at periods when such were masked on the Royal Sovereign indicators, and the infected plants rapidly succumbed.

B. CULTIVATED VARIETIES OTHER THAN ROYAL SOVEREIGN

The five leading commercial varieties selected for the initial experiment were found to form a susceptibility series between the two extremes described above. At the *F. chiloensis* end came Lefebvre, which approximated to this species closely in high symptomless-carrier capacity and in high (although not absolute) resistance. Towards the *F. virginiana* end (high symptom expression and susceptibility), came Sir Joseph Paxton and at the extreme end, Royal Sovereign itself.

The initial stages of the experiment were carried out in the greenhouse, but from the fall of 1935 throughout 1936 the complete series was transferred to the open air. The *relative* varietal reaction to Yellow-edge remained the same under both sets of conditions.

C. "CRINKLE" AT EAST MALLING ON ROYAL SOVEREIGN, AND *F. vesca* AS AN INDICATOR

In 1934, Ogilvie, Swarbrick and Thompson (8) published a description of symptoms observed on Royal Sovereign in the western districts of England, answering closely to Zeller and Vaughan's descriptions of the virus disease "Crinkle" in the Pacific northwestern states (13).

Shortly after the writer's return from Canada (in August, 1934) similar symptoms were found at East Malling on seedlings of certain crosses of English and American varieties, under field test. In collaboration with the Long

Ashton Horticultural Research Station, attempts to transmit the symptoms (by runner-inarching), from these seedlings and from seedling type-material supplied by Long Ashton, yielded positive results. At a later stage, however, all the indicator plants in this experiment developed definite Yellow-edge symptoms, superimposed to varying degree on the "Crinkle" symptoms. The distinct nature of the "Crinkle" disease, however, became evident from results of an experiment in infection with Yellow-edge of the common woodland strawberry, *Fragaria vesca*. Normal *F. vesca* plants runner-inarched to Yellow-edge Royal Sovereign rapidly developed symptoms typical of Yellow-edge. In addition to these symptoms, however, a distinct blistering of the leaves, not observed in similar infections of other species and varieties, was recorded on the test plants. On the other hand, contrary to expectation, similar ("Crinkle" type) symptoms, *but uncombined with the Yellow-edge symptoms*, developed on a single *F. vesca* plant inarched to a "normal" (Yellow-edge-free) Royal Sovereign indicator plant, suggesting the infection of the latter with a disease distinct from Yellow-edge. On close examination of all available Yellow-edge and "normal" Royal Sovereign plants, small and generally very inconspicuous groups of minute circular chlorotic lesions were found, invariably on the former and on a large proportion of the latter.

In 1936, a large-scale confirmatory experiment was carried out testing *F. vesca* as an indicator of Yellow-edge and "Crinkle". A considerable number of "normal" Royal Sovereign plants derived from the clonal family supplying the indicators in the present investigation, were runner-inarched singly to "normal" *F. vesca* plants. Clearly marked and in many cases severe symptoms of the "Crinkle" type, entirely uncomplicated by those of the Yellow-edge type, subsequently developed on 78% of the latter. On the other hand, up to the present (winter 1936-37) all the Royal Sovereign plants have remained uniformly "healthy", vigorous, and normal in appearance, with the exception of the aforementioned inconspicuous groups of minute chlorotic spots. In other clones of Royal Sovereign, distinct in origin from that used in the above experiments, a proportion of the *F. vesca* indicators developed clearly marked Yellow-edge symptoms in addition to the "Crinkle" symptoms.

It has thus become evident that in addition to Yellow-edge, a further distinct virus disease ("Crinkle") is widely, and probably invariably, combined with the Yellow-edge disease in Royal Sovereign, and is widely distributed, generally in "mild" and inconspicuous form, throughout the main Yellow-edge-rogued, "normal" clonal family, and other "healthy" stocks of this variety at East Malling.

Making allowance for a probable spread of infection during the short period between the shipments to St. Catharines and the above experimental survey, it can be assumed, therefore, that the majority of the indicator plants used in the 1934-35 experiments (V, above) and probably in the 1933 experiments were infected with "mild Crinkle".

The great majority of clonal "normal" Yellow-edge-free Royal Sovereign plants hitherto observed in the field, and all plants used as indicators in

the greenhouse, have shown "Crinkle" only in the "mild" and comparatively innocuous form, *i.e.*, the plants are uniformly vigorous and normal, except for inconspicuous groups of minute circular lesions detectable only on close examination. "Serious" cases as illustrated by Ogilvie, Swarbrick and Thompson (8) were identified in 1935 and more commonly in 1936, their distribution in plantations being quite sporadic. That the "serious" phase of "Crinkle" is the result of a re-infection of plants with a further virus pathogen, *i.e.*, that "Crinkle" is complex in origin, is indicated by the results of experiments in 1935, when the "serious" phase of "Crinkle" was artificially induced on Royal Sovereign plants infected with "mild Crinkle" by inarching such plants (i) with seedling varieties infected with "serious Crinkle" plus Yellow-edge, and (ii) with plants of the Stirling Castle variety proved to be free from Yellow-edge and with no visible symptoms of "Crinkle". In the former instance, Yellow-edge as well as "serious Crinkle" was transmitted to the "mild-Crinkle"-infected indicators. Such results provide further evidence (1) that Yellow-edge and "Crinkle" are distinct in origin and (2) that "serious Crinkle" is induced by the interaction of two or more viruses.

General Discussion

The results obtained in the present investigations have already been discussed in considerable detail. The following general considerations and conclusions, with particular reference to the bearing of the results of the recent experiments at East Malling on those of the St. Catharines experiments call, however, for review.

Contrary to what might have been expected from field surveys of symptoms, the experiments with three commercial varieties grown in Ontario have further indicated the close similarity of the Yellow-edge disease in southeast England to the analogous disease in southern Ontario, and have provided considerable evidence in favor of their possible identity. The relationship, however, between either of these diseases and Xanthosis in California, has not yet been experimentally determined so that, pending further detailed and experimental comparisons of the three diseases, they must merely be regarded as common members of a well defined group of virus diseases.

It has further been shown that the three local varieties in question, Parson's Beauty, Premier (Howard 17), and Glen Mary (and to an outstanding degree the last two varieties) possess markedly the "symptomless-carrier" capacity, and that consequently the paucity or the entire absence of diagnostic symptoms in field plantations of these varieties in Ontario does not imply that the incidence of the Xanthosis-Yellow-edge type of disease is slight or that the disease does not occur.

Experimental evidence further indicates that the apparent contrast in the general symptomatological picture in Ontario and in southeastern England is related primarily to inherent differences in susceptibility between the prevalent varieties in the two countries and to differences in cultural conditions mainly in so far as such determine the varieties selected for local cultivation.

The recent experiments at East Malling (1935) on the reaction to Yellow-edge of varieties other than Royal Sovereign, have further shown that this contrast in the symptomatological pictures is not, generally speaking, as great as was suggested when Royal Sovereign was the sole representative of the English varieties available for comparison. Certain widely grown varieties such as Lefebvre and Oberschlesien were found to exhibit a high resistance and symptomless-carrier capacity comparable to that of the three Ontario varieties investigated at St. Catharines.

The close association of high resistance to the action of Yellow-edge with the symptomless-carrier capacity was further shown by these experiments to be referable to the contrasted reaction to the disease of the two parent species, common to both the English and Canadian cultivated strawberry varieties, namely *F. chiloensis*, which up to the present has proved to be a perfect symptomless-carrier of Yellow-edge with a markedly high degree of resistance which may prove to be absolute, and *F. virginiana*, which combines very high susceptibility with continuous and vivid symptom expression. The relative degree to which such varieties as Premier, Glen Mary, Lefebvre and Oberschlesien approximate to *F. chiloensis* in resistance to the deteriorating action of virus is a subject for future investigation. Certain of these varieties, however, have shown clearly that their high resistance is not absolute and as these varieties do not appear to exhibit diagnostic symptoms sufficiently clearly to allow of an adequate elimination of infected plants in propagating beds, and thus of the maintenance of Yellow-edge-free stocks, their survival in commerce would appear to be of limited duration.

Finally, the recent experimental evidence, (a) of a wide infection of the Yellow-edge-free Royal Sovereign indicator plants used in the present investigation with a "mild" form of disease distinct in origin from Yellow-edge and conforming closely to descriptions of virus disease of the "Crinkle" type, and (b) the complex origin of the "serious" phase (by re-infection of a "mild Crinkle" infected plant) provides an explanation of the anomalous behavior of the Premier test plants in graft-series V and VI. It would appear that the race of Premier plants used in these series, *i.e.*, that raised at the St. Catharines laboratory was, in addition to Yellow-edge, infected with a further virus which in combination with the "mild Crinkle" virus contributed by the Royal Sovereign indicators, causes the former plants to pass into a condition of rapid deterioration. Verification of this hypothesis must await further work in the analysis of the virus complex in strawberries; meanwhile the explanation of the deterioration of the Premier plants in question as due primarily to a reciprocal infection of these plants from the "mild Crinkle"-infected Royal Sovereign indicators is the simplest yet available.

It yet remains to investigate the difference in virus content between the Premier plants in Series V and VI, and those of the previous and subsequent series, a difference which must be assumed if the above hypothesis is accepted. Meanwhile further evidence is provided to show that stocks of Premier runners from different sources may, in spite of a general uniformity of appearance, differ considerably in virus content.

Acknowledgments

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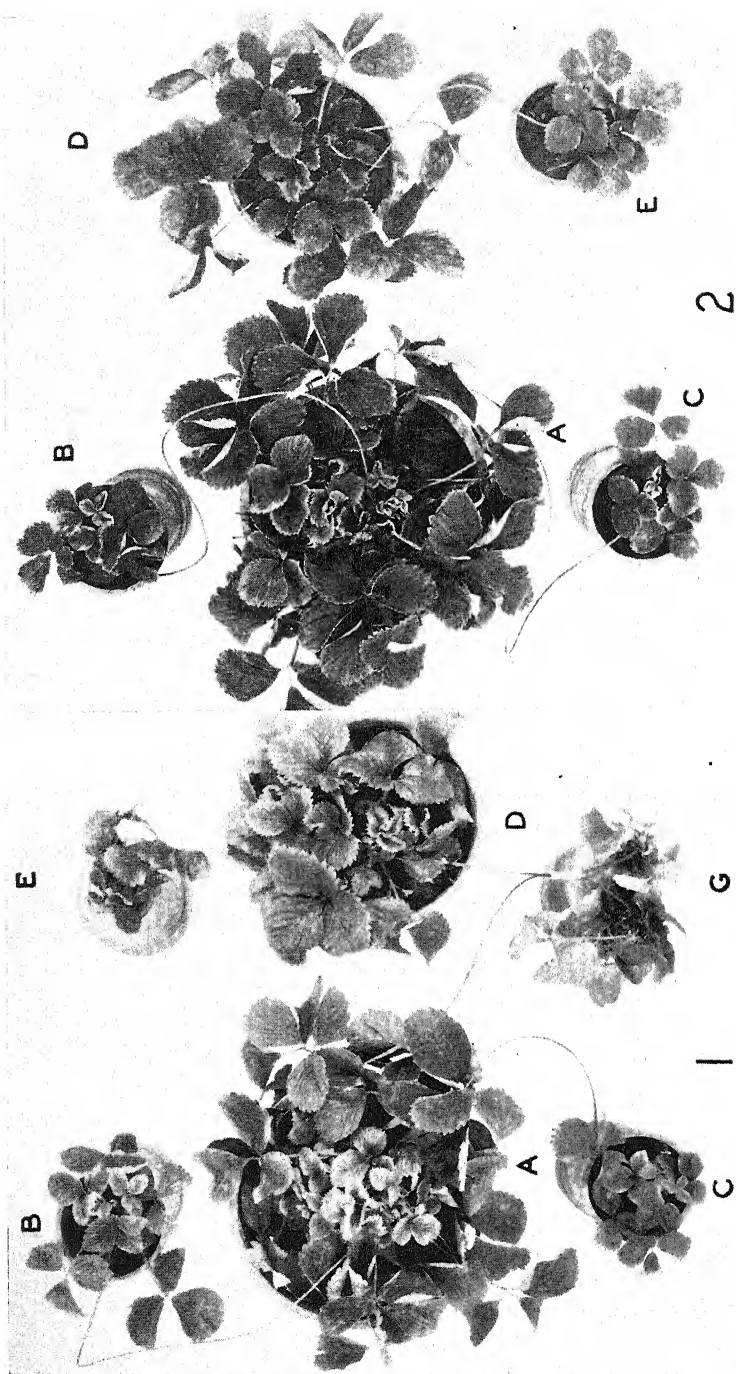
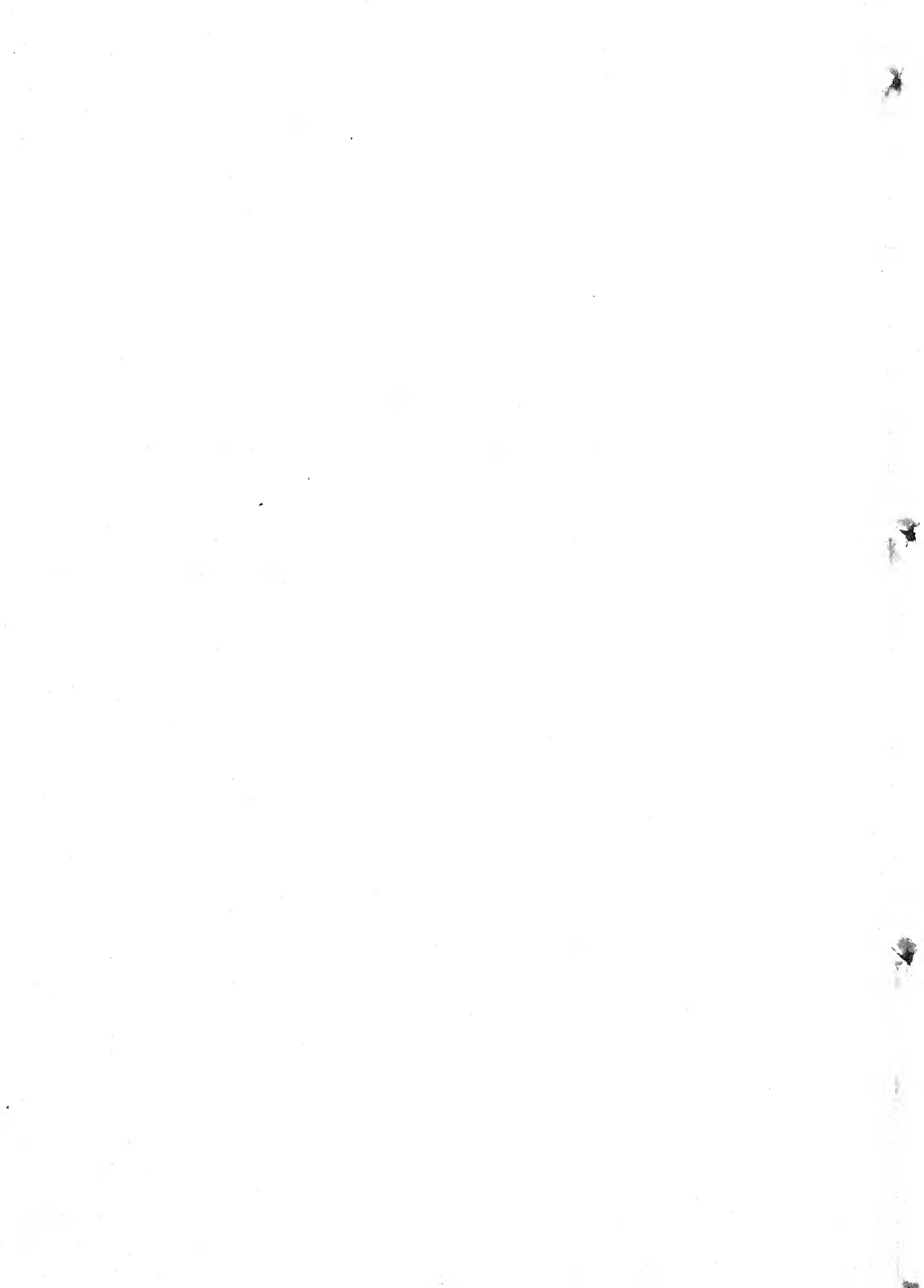


FIG. 1. Graft-unit of variety Parson's Beauty set up 5.8.33. Photo. 30.10.33. A, Indicator plant with B, C, supplementary runners. D, Test plant with E, supplementary runner. G, Grafted stolons showing point of union. FIG. 2. Graft-unit of variety Premier set up 5.8.33. Photo. 30.10.33. Lettering as above. Grafted stolon previously removed.



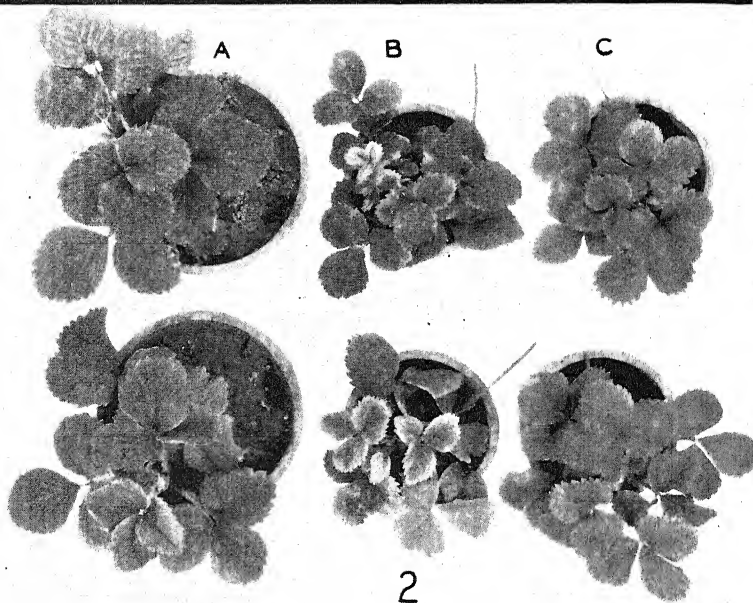
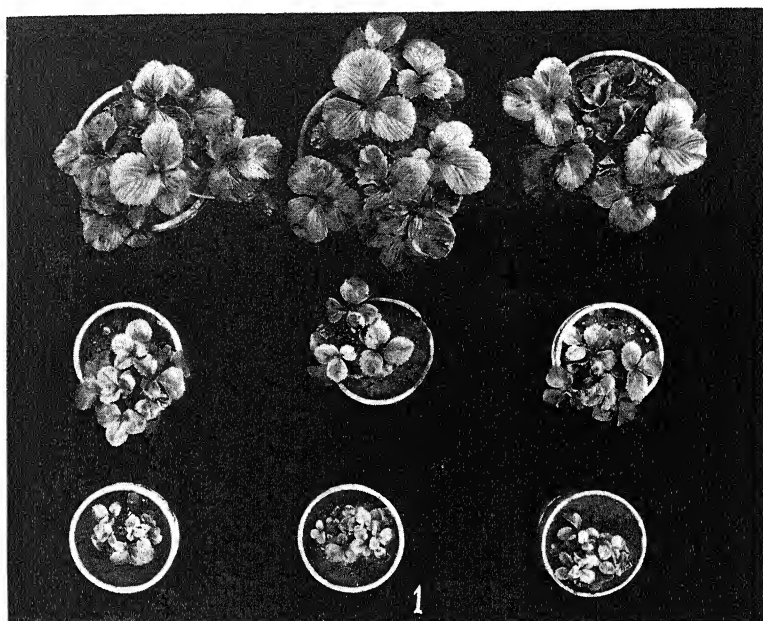
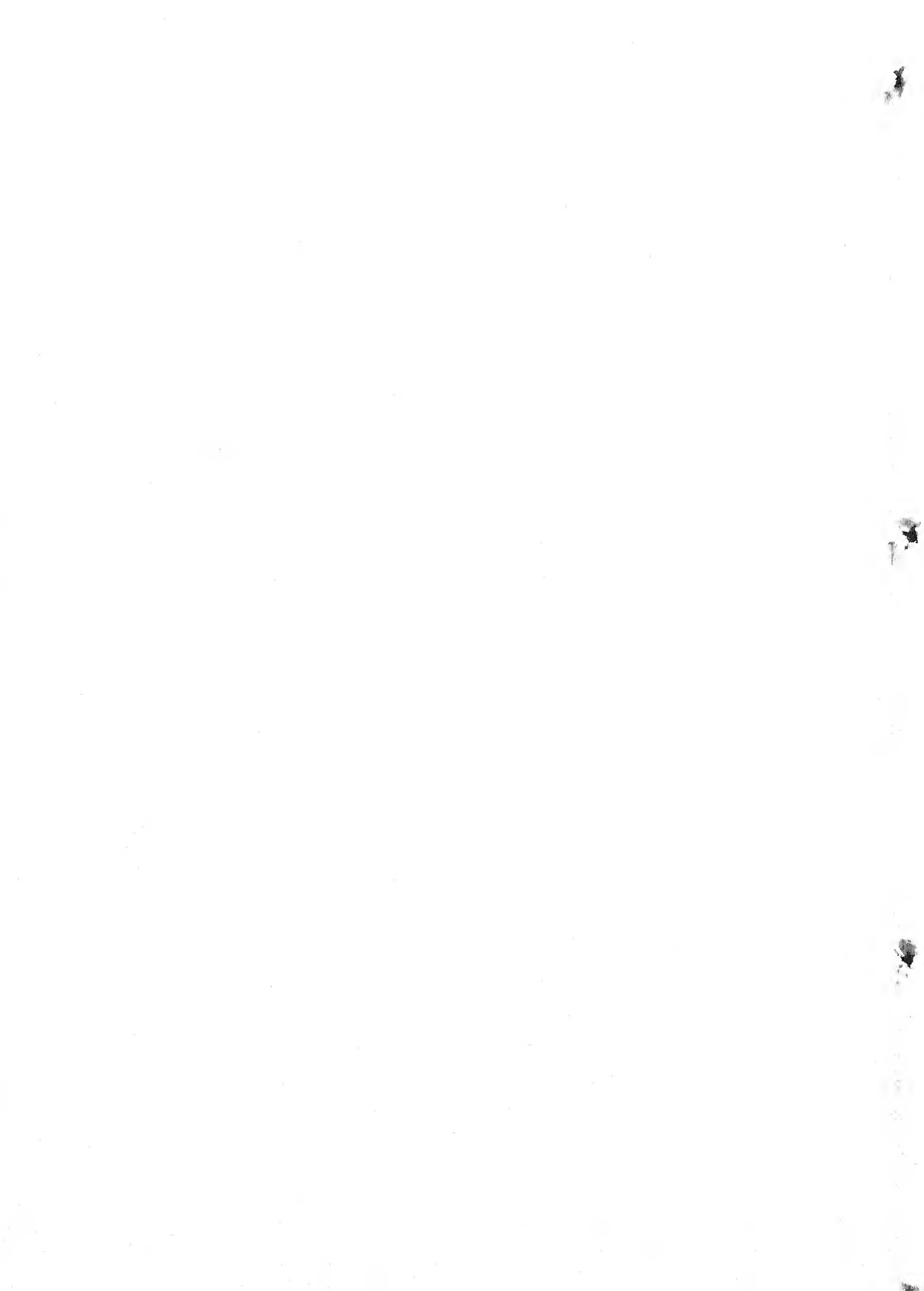
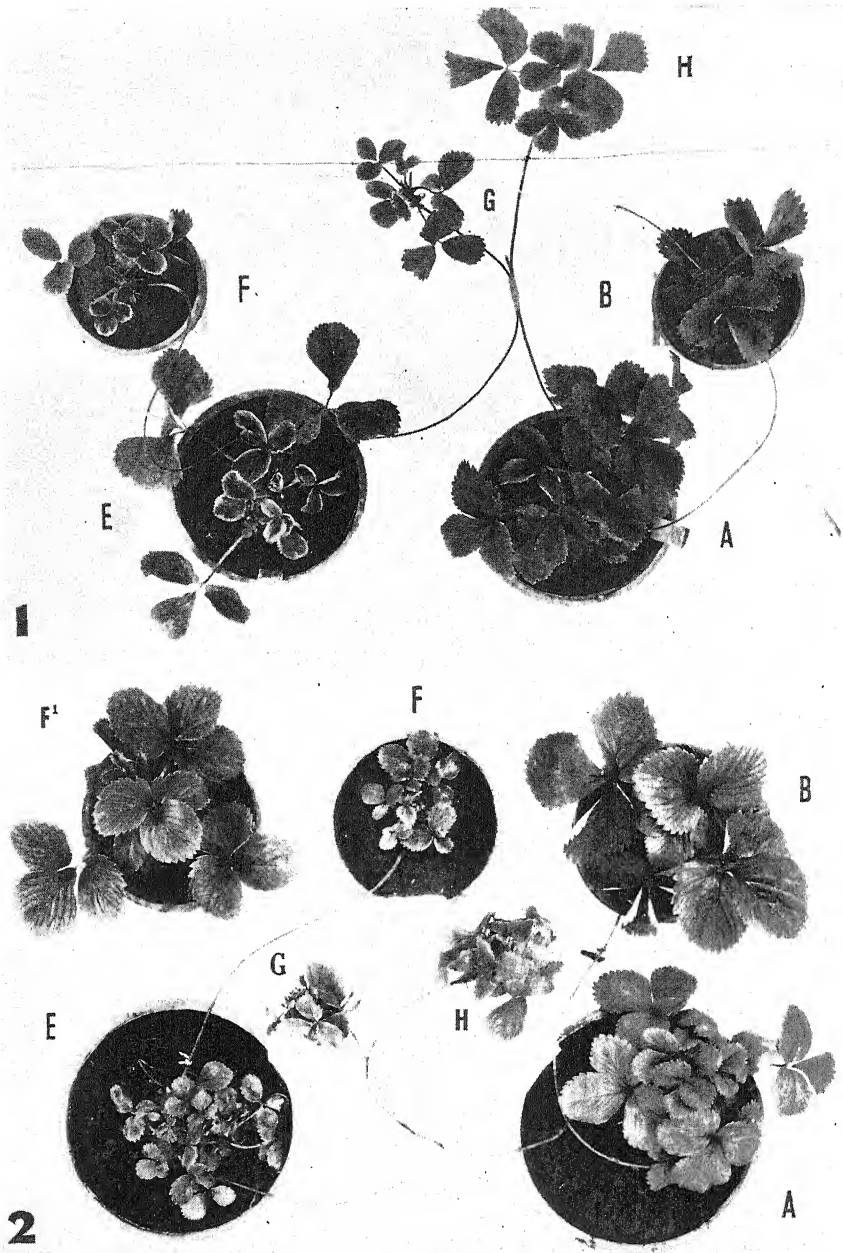


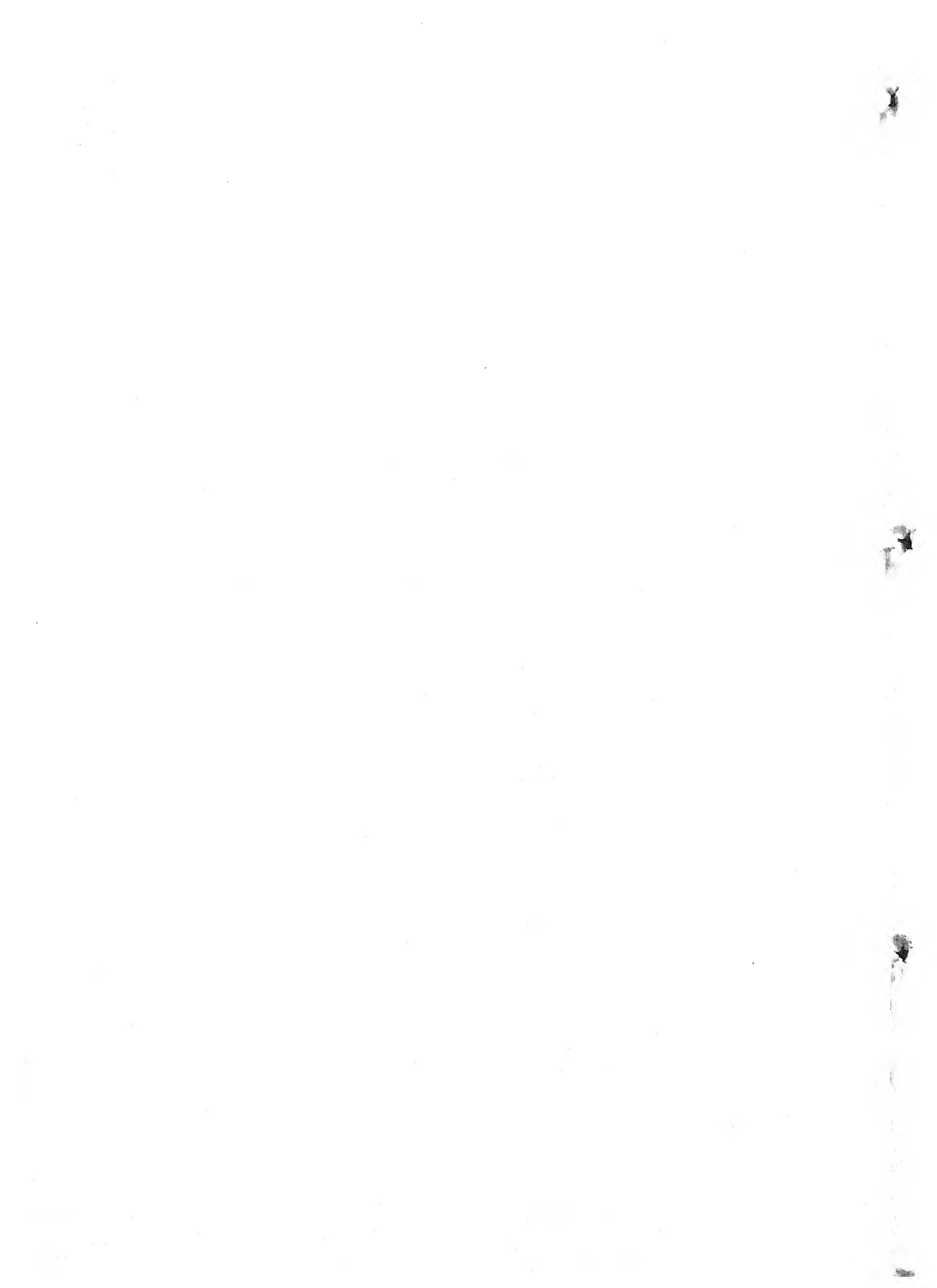
FIG. 1. *Royal Sovereign* runner plants. Top row, from ungrafted controls. Middle row, from Premier graft-units. Bottom row, from Parson's Beauty units. Photo. 9.3.34.

FIG. 2. Runner plants. A, *Royal Sovereign* from ungrafted control. B, *Royal Sovereign* from Premier graft-units. C, Premier from the same graft-units.





FIGS. 1 AND 2. *Glen Mary* \times *Royal Sovereign* graft-unit showing the condition of the component plants six and one-half weeks (Fig. 1) and four and one-half months (Fig. 2) subsequent to grafting. A, B and H, *Glen Mary* parent, runner in autoclaved soil and grafted runner, respectively. E, F, F' and G, *Royal Sovereign* parent, runner in autoclaved soil, non-grafted sister-runner of F (approximately the same age) in autoclaved soil and grafted runner, respectively.



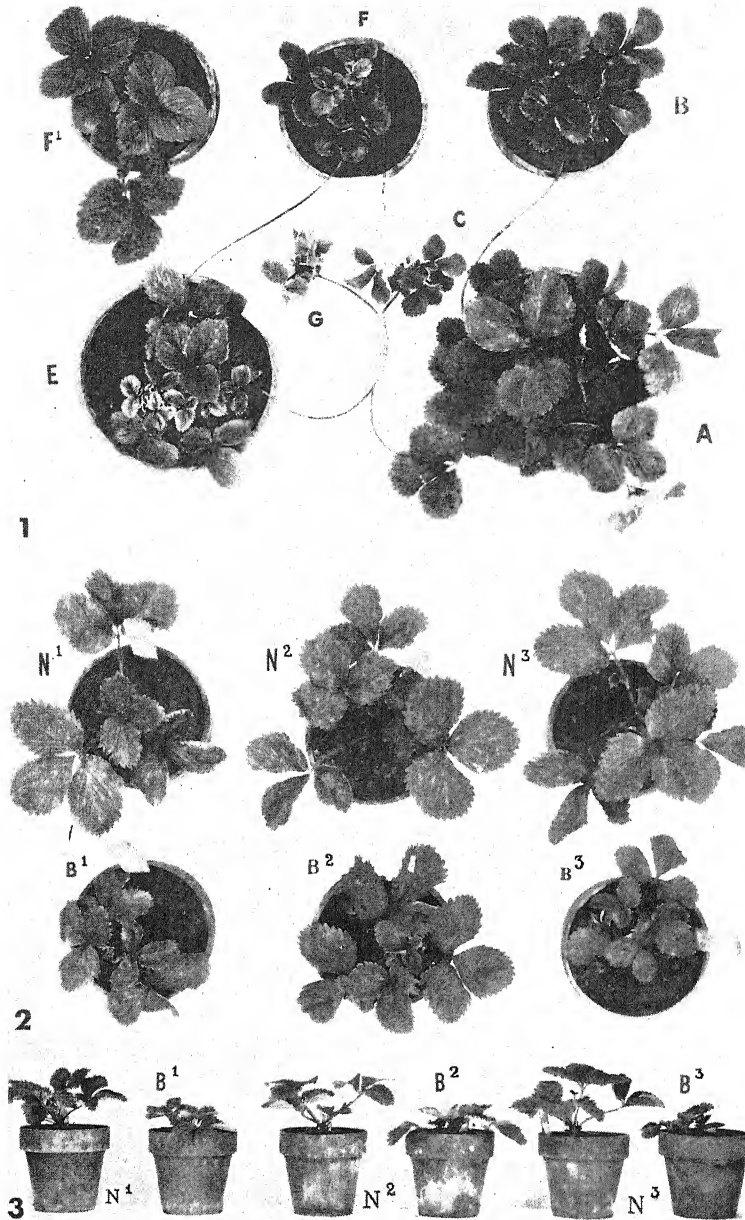


FIG. 1. *Premier* × *Royal Sovereign* graft-unit showing the condition of the component plants ten weeks subsequent to grafting. A, B and C, *Premier* parent, runner in autoclaved soil and grafted runner, respectively. E, F, F' and G, *Royal Sovereign* parent, runner in autoclaved soil, non-grafted sister-runner of F (approximately the same age) in autoclaved soil and grafted runner, respectively. FIGS. 2 AND 3. Surface and lateral views, respectively, of genetically identical pairs of *Premier* runner plants, the "N" components of each pair having been detached from the parent previous to the latter being runner-grafted with *Royal Sovereign*, the "B" component of each pair having been left attached to the parent subsequent to grafting.

A CYTOLOGICAL STUDY OF THE GENUS *POA* L.¹

By J. M. ARMSTRONG²

Abstract

The somatic chromosome numbers of 20 species of *Poa* were determined. The basic chromosome number for the genus was found to be seven. The species arranged themselves in a polyploid series from diploid to dodecaploid, tetraploids and hexaploids being the most numerous. Three aneuploid species possessed chromosome numbers suggestive of a nonaploid origin. Polymorphism was found to be present in *P. compressa* L., *P. palustris* L. and *P. nemoralis* L. All species examined conformed to the long chromosome type common to the subfamily, Pooideae. The spindle fibre attachment for the chromosomes in the various species ideograms was found to be regularly median or submedian.

The chromosome variability and the mode of seed production were examined in *Poa pratensis* L., using selected, uniform strains, indigenous plants and plants grown from commercial seed. The somatic chromosome number was found to range from 50 to 87 ± 1 , 10 of the 19 plants examined possessing aneuploid numbers. The selected strains possessed the same chromosome number for both plants examined, while in the other material the number was variable. A study of meiosis in the P.M.C. showed the selected strains to vary from regular behavior to an irregularity of 3.9 unpaired univalents per cell. All strains possessed large percentages of morphologically good pollen which germinated actively on the stigmas. Reduction was observed in the E.M.C. of the selected strains and a study of the course of embryological development showed no irregularities which might lead to aposporous reproduction. A high frequency of polyembryony was observed which was correlated to the degree of irregularity at meiosis. A theory is advanced to explain how constant aneuploid numbers may be maintained in sexually reproduced strains.

1. SOMATIC CHROMOSOMES

Poa belongs to the large tribe Festuceae of the Gramineae. The genus has a wide distribution, from moist tropical conditions through every type of habitat in temperate regions to alpine conditions in mountain ranges. There are about 200 recognized species. These species are known as meadow grasses in Britain and as bluegrasses in America. They are mostly perennials but there are a few annuals. Some are stoloniferous while others are of the bunch grass type. While *Poa* is regarded taxonomically as one of the primitive genera of grasses, it presents many difficulties in classification.

Cytologically the genus has not been very extensively investigated. Eleven species were examined by Stahlin (14), nine by Avdulov (2, 3) and six by Müntzing (9). Allowing for repetition, 12 species in all have been examined. This was sufficient to establish the polyploid nature of the genus, with seven as the basic chromosome number. The existence of polymorphic species, characterized by different chromosome numbers for the different biotypes has also been discovered. Müntzing investigated in considerable detail the polymorphous species, *P. alpina*, and to a lesser extent *P. pratensis*. Biotypes were studied which possessed constant aneuploid chromosome numbers, which the author believed were due to asexual seed formation.

Twenty species of *Poa* were examined in the present study. These include 11 not hitherto reported upon. It was thought that a cytological examination

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of this group, fairly representative of the genus as regards ecological distribution, would enable some conclusions to be drawn as to the nature and origin of polyploidy in the genus. The possibility of apomictical seed formation in *P. pratensis* has also been investigated. The report of this investigation will be dealt with in a separate section of this paper.

MATERIALS AND METHODS

The plant material used was taken from the introduction nursery of the Division of Forage Plants, Central Experimental Farm, Ottawa, where the economic possibilities of various species are being investigated. The original source of the material is given in Table I. The correct naming of the various species was determined by the Division of Botany, Central Experimental Farm, and in the case of certain European introductions by the Royal Botanic Gardens, Kew, England.

The somatic chromosome studies have all been made on root tips of plants grown in the greenhouse. These plants were taken as clones from plants in the nursery, which in turn were established from seed. Root tips were fixed in La Cour's (6) 2 B. E. fixative. Sections were cut 12 μ thick and stained by Newton's iodine gentian violet method. Drawings were made with the aid of a camera lucida, 30 \times ocular and Leitz objective 1.5 mm., N. A. 1.3, giving a magnification at table level of approximately 6400 \times . This has been reduced in reproduction to approximately 2150 \times .

Observations

In Table I, a list of the species examined, their source and chromosome number is given. The nomenclature of species native to or introduced to America is that given by Hitchcock (5).

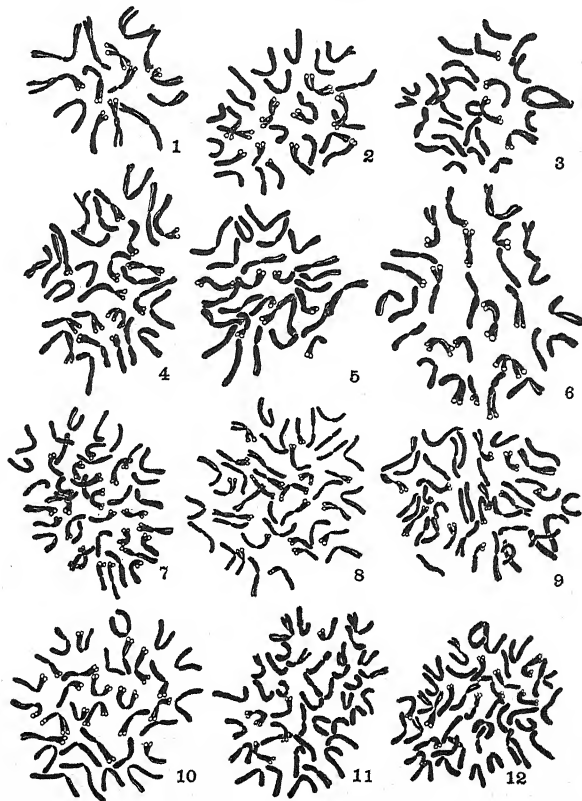
TABLE I
LIST OF SPECIES EXAMINED, SOURCE AND SOMATIC CHROMOSOME NUMBERS

Species	Source	No. of plants examined	Chromosome numbers
<i>P. trivialis</i> L.	Vilmorin Andrieux, Paris	2	14
<i>P. palustris</i> L.	Vilmorin Andrieux, Paris	2	28
<i>P. palustris</i> L.	University of Manitoba	2	28
<i>P. annua</i> L.	Vilmorin Andrieux, Paris	2	28
<i>P. bulbosa</i> L.	Woronesh, U.S.S.R.	2	28
<i>P. badensis</i> Haenke.	Woronesh, U.S.S.R.	2	28
<i>P. macrantha</i> Vasey	Soil Erosion Service, U.S.A.	2	28
<i>P. nemoralis</i> L.	Sutton & Sons, England	2	42
<i>P. compressa</i> L.	Ewings Seed Co., Montreal	2	42
<i>P. compressa</i> L.	Woronesh, U.S.S.R.	2	56
<i>P. ochroleuca</i> Stern.	Woronesh, U.S.S.R.	2	42
<i>P. alpina</i> L.	Woronesh, U.S.S.R.	3	32 - 34
<i>P. bodryoides</i> L.	Woronesh, U.S.S.R.	2	42
<i>P. sterilis</i> L.	Woronesh, U.S.S.R.	2	42
<i>P. conferta</i> Blyth	Woronesh, U.S.S.R.	2	56
<i>P. epilis</i> Scribn.	Soil Erosion Service, U.S.A.	2	56
<i>P. confusa</i> Rydb.	Dr. S. E. Clarke, Manyberries, Alberta	4	62
<i>P. ampla</i> Merr.	Soil Erosion Service, U.S.A.	2	64
<i>P. nevadensis</i> Vasey	Soil Erosion Service, U.S.A.	2	62
<i>P. arctica</i> R. Br.	Dr. O. M. McConkey, Ontario Agr. College	2	70
<i>P. scabrella</i> (Thurb.) Benth.	Soil Erosion Service, U.S.A.	2	84

P. trivialis L. Introduced from Europe to America where it is now widely distributed in moist situations. The plants examined were from a European commercial seed source and proved to be diploid, $2n = 14$ (Fig. 1). This count was also obtained by Stahlin, Avdulov and Müntzing. *P. chaixii* L. (= *P. sudetica* Haenke.) is the only other diploid species so far identified by the above investigators.

P. palustris L. Hitchcock considers that this species is probably indigenous to both Europe and America, since it is widely distributed in both continents. It is a loosely tufted species found in moist habitats. Plants from two sources were examined cytologically and both proved to be tetraploids, $2n = 28$ (Fig. 2). This agrees with the count obtained by Avdulov. Stahlin, however, reported a hexaploid, $2n = 42$. While *P. palustris* is generally regarded as a "good" species, it shows considerable morphological variability, and polymorphic forms, differing in their chromosome numbers, apparently exist.

P. annua L. This annual species was introduced into America from Europe. The material examined was from a European source. It proved



FIGS. 1-12. FIG. 1. *Poa trivialis* L., $2n = 14$. FIG. 2. *Poa palustris* L., $2n = 28$. FIG. 3. *Poa annua* L., $2n = 28$. FIG. 4. *Poa bulbosa* L., $2n = 28$. FIG. 5. *Poa badensis* Haenke., $2n = 28$. FIG. 6. *Poa macrantha* Vasey $2n = 28$. FIG. 7. *Poa nemoralis* L., $2n = 42$. FIG. 8. *Poa compressa* L., $2n = 42$. FIG. 9. *Poa ochroleuca* Stern., $2n = 42$. FIG. 10. *Poa alpina* L. $2n = 33$. FIG. 11. *Poa bodryoides* L., $2n = 42$. FIG. 12. *Poa sterilis* L., $2n = 42$.

to be a tetraploid $2n = 28$ (Fig. 3). This number agrees with that found by Stahlin and Avdulov.

P. bulbosa L. Introduced from Europe to America where it has become widely distributed. Its distinguishing morphological feature is the bulblets which are found in place of normal flowers. Plants from one source were examined and proved to be tetraploid, $2n = 28$ (Fig. 4).

P. badensis Haenke. (*P. alpina* L. var. *badensis* Koch). A European species introduced from the U.S.S.R. This species proved to be a tetraploid, $2n = 28$ (Fig. 5). Stahlin reports $2n = 42$ for this species.

P. macrantha Vasey. A dioecious species, indigenous to the Pacific Coast, its habitat being the saline sand dunes along the coast. This species is a tetraploid, $2n = 28$ (Fig. 6).

P. nemoralis L. Introduced from Europe to America and now widely distributed in meadow habitats. Our material was obtained from an English commercial source and proved to be hexaploid, $2n = 42$ (Fig. 7). This number agrees with that observed by Stahlin. Avdulov, however, found a tetraploid form and Müntzing an octoploid. Biotypes apparently exist in this species, which show intraspecific variation in chromosome number, probably of autopolyploid origin, since the different biotypes are not taxonomically distinct.

P. compressa L. Introduced from Europe, according to Hitchcock, although it is commonly known as Canada bluegrass. It is characterized by strongly flattened stems. It thrives on certain heavy, clay soils in western Ontario and is often the dominant species on light sandy soils of poor fertility. Our material was obtained from two sources. That from a local commercial source proved to be hexaploid, $2n = 42$ (Fig. 8), while that introduced from the U.S.S.R. proved to be octoploid.* Stahlin and Müntzing found their material to be hexaploid while Avdulov reported an octoploid. Polymorphism is apparently present in this species also, although the autopolyploid origin is not so definite as in *P. nemoralis*, since the variability in the polymorphic forms is more marked.

P. ochroleuca Stern. var. *submoniliformis* Makino. Obtained from Woronesh, U.S.S.R., but the taxonomic description could not be traced by the Royal Botanic Gardens, Kew. In many respects the species closely resembles *P. nemoralis* L. It proved to be a hexaploid, $2n = 42$ (Fig. 9).

P. alpina L. Indigenous to the whole northern hemisphere, its habitat outside of the Arctic regions being mountain meadows. Three plants, examined cytologically from a European introduction, possessed somatic chromosome numbers of 32, 33 and 34 (Fig. 10). Stahlin reported a biotype which had $2n = 42$. Müntzing found quite a complex situation in the Swedish

* Mr. W. Dore, taxonomist at the Botany Division, Central Experimental Farm, Ottawa, has the following to say about this form: "This is a most peculiar plant in that the florets are devoid of any trace of pubescence such as is typical of this species. Every one of the 23 Canadian specimens and four Scandinavian specimens I have examined is typical in being appressed-pilose on the keel and marginal nerves of the lemma, with webby hairs at the base. In this specimen the lemmas are entirely glabrous (except for a slight scabiosity on the upper part of the keel). It is similar to typical *P. compressa* with regard to all the other characters, and I do not think it can be any other species."

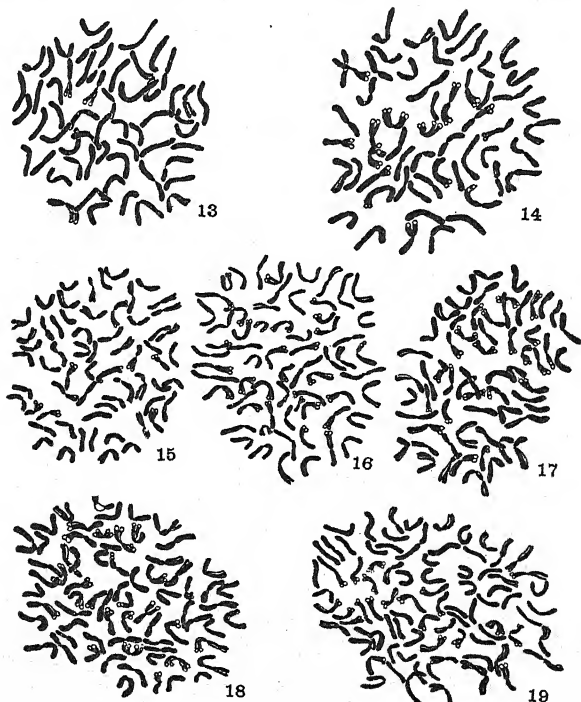
and Swiss biotypes which he examined. Two Swedish biotypes had constant aneuploid chromosome numbers of 33 and 38 and showed a high degree of morphological uniformity within each biotype. The Swiss biotypes, on the other hand, were found to be quite variable in the chromosome numbers, ranging from 22 to 34, and displayed a high degree of morphological variability. Müntzing considered the latter to be unstable sexual types and the former to be reproduced apomictically. The plants in our material varied considerably in vigor and this fact, in conjunction with the variation in chromosome number, indicates that this form is probably an unstable sexual type.

P. bodryoides L. Introduced from Woronesh, U.S.S.R. but has not been identified with certainty by our taxonomists. It proved to be a hexaploid, $2n = 42$ (Fig. 11).

P. sterilis L. Introduced from Woronesh, U.S.S.R. and its correct naming is still uncertain. The plants examined proved to be hexaploid, $2n = 42$ (Fig. 12).

P. conferta Blyth. Introduced from Woronesh, U.S.S.R. The plants examined were octoploids, $2n = 56$ (Fig. 13).

P. epilis Scribn. Found in the Pacific Coast area, its habitat being mountain meadows above the timber line. It is characterized by a condensed ovoid panicle. The species proved to be an octoploid, $2n = 56$ (Fig. 14).



FIGS. 13-19. FIG. 13. *Poa conferta* Blyth., $2n = 56$. FIG. 14. *Poa epilis* Scribn., $2n = 56$. FIG. 15. *Poa confusa* Rydb., $2n = 62$. FIG. 16. *Poa ampla* Merr., $2n = 64$. FIG. 17. *Poa nevadensis* Vasey., $2n = 62$. FIG. 12. *Poa arctica* R. Br., $2n = 70$. FIG. 19. *Poa scabrella* (Thurb) Benth., $2n = 84$.

P. ampla Merr. Occurs in the Pacific Coast area in meadows and rocky slopes from New Mexico to the Yukon territory. Typical forms are robust and more or less glaucous. The chromosome number of the plants examined was aneuploid, $2n = 64$ (Fig. 16).

P. confusa Rydb. While Rydberg gives *P. confusa* a species ranking, Hitchcock considers it to be a form of *P. ampla*. It is common on the eastern part of the range of *P. ampla* and is distinguished from it by being smaller and non-glaucous. The four plants examined (collected by Dr. S. E. Clarke, Manyberries, Alta.) proved to be aneuploid, $2n = 62$ (Fig. 15). Cytologically it is not quite identical with *P. ampla* although the chromosome number suggests the same origin.

P. nevadensis Vasey. Range of distribution from the Yukon territory south to California with the greatest concentration in Montana and eastern Washington. A small isolated colony has also been discovered in Maine. Cytologically it was found to be aneuploid, $2n = 62$ (Fig. 17).

P. arctica R. Br. Range of distribution from Arctic regions south to Nova Scotia and found also on the slopes of the Rocky Mountains above the timber line where the ground is bare of snow only three months in the year. Our material was found to be decaploid, $2n = 70$ (Fig. 18).

P. scabrella (Thurb.) Benth. Occurs in meadows and open woodlands at low to medium altitudes at the Pacific coast. It is distinguished morphologically by the scabrous character of culms and leaves. Aside from certain biotypes of *P. pratensis*, it proved to have the highest chromosome number ($2n = 84$) of any species examined (Fig. 19).

DISCUSSION

In recent years there has been a wide application of cytology to systematics. This has been due largely to improved methods of fixation and staining, permitting a closer observation of chromosome detail. The chromosome characters of value in application to systematics are basic chromosome numbers, polyploidy, chromosome length, the position of the spindle-fibre attachment and secondary features such as the presence of heads and trabants.

Lewitsky (7) has ably reviewed the use of the karyotype in systematics. The author uses the term, karyotype, to denote a complex of nuclear characters keeping its significance now over individual, then over race, species, genus, etc. He designates the graphical representation of a karyotype as an ideogram. Separate species would be expected to have characteristic and constant ideograms, and the ideograms of all species within a genus should possess certain features in common. The author points out the danger of too rigid a karyotypic interpretation since closely related species taxonomically may differ widely in their ideograms, owing to the operation of chromosome translocation, and conversely species widely separated taxonomically may show a fairly close similarity in their ideograms. But on the whole a parallelism of karyotypical and external plant characters holds.

The basic chromosome number of the genus *Poa* is seven, which is the most common basic number not only for the tribe Festuceae but for the Gramineae as a whole. Most of the species examined are even multiples of this basic number and arrange themselves in a polyploid series from $2x$ to $12x$ (where x is the basic number). The frequency distribution for the series is one diploid, six tetraploid, five hexaploid, three octoploid, one decaploid and one dodecaploid.

The species, *P. ampla*, *P. confusa* and *P. nevadensis*, constitute exceptions to regular polyploidy. Their aneuploid chromosome numbers of 62 and 64 suggest that they are derivatives from a hybrid nonaploid ($2n = 63$) and have become stabilized by the loss or duplication of a chromosome. If these aneuploid numbers are constant for the various species (this may be presumed to be the case for *P. confusa* in which four plants were examined) either the meiotic behavior is fairly regular, or the chromosome constancy is due to the operation of apomixis, as Müntzing has suggested for certain biotypes of *P. alpina* and *P. pratensis*. Opposed to this hypothesis is the well known difficulty in classification of a group of western Poas comprising such species as *P. canbyi*, *P. nevadensis* and *P. confusa*. This difficulty may be due to hybridization within the above species or to a high degree of heterozygosity. Such difficulties in classification would not be met with if the species were reproduced apomictically.

The situation in *P. alpina* has been fully discussed by Müntzing, and similar biotypes to the one which we have examined were studied by Müntzing and Avdulov.

Next in importance to basic chromosome number and polyploidy in applying karyotology to systematic studies is chromosome length. Frequently tribes in a family are characterized by a common diminution in chromosome length. For example the subfamily Panicoideae, in the Gramineae, has small short chromosomes in comparison to the other subfamily Pooideae, in which the chromosomes are comparatively large and long (Avdulov (2)). Analogous to this situation is the difference in chromosome length in the tribes Viciaeae and Trifolieae in the Leguminosae. The former is characterized by large chromosomes and the latter by small ones. The genus *Poa* conforms to the long chromosome type common to the subfamily Pooideae. Some variation in chromosome length exists throughout the various species ideograms of the series. The shorter chromosomes are approximately one-half the length of the longer chromosomes, with others intermediate in length.

The spindle fibre attachment for the chromosomes in the various ideograms of the genus *Poa* is in general median or sub-median. Occasionally chromosomes were observed in which the attachment is subterminal (Figs. 1, 4). Lewitsky (7) on the basis of his study of the Helleboreae concluded that there has been a progressive shortening of one arm in many of the chromosomes, primitive members of group having more isobrachial chromosomes and derived members, mostly heterobrachial ones. Since there is no marked increase in the proportion of heterobrachial chromosomes in the higher polyploids of the

genus it would indicate that their origin, whether due to auto- or allopolyploidy, from lower polyploids in the series, has stimulated very little secondary differentiation such as might be brought about by chromosomal interchange. This conclusion, however, must be regarded as tentative until meiotic studies are completed.

Concerning the origin of the higher polyploids of the genus *Poa*, we are inclined to believe that both auto- and allopolyploidy have taken place in different instances. In *P. nemoralis* and *P. compressa* the existence of polymorphic forms with different chromosome numbers has been noted. As these forms were not sufficiently different taxonomically to give species ranking, the higher polyploid in the species probably originated from the lower in an autopolyploid manner. In the case of decaploid and dodecaploid species, their origin was more likely due to allopolyploidy—the hybridization of lower members of the series, followed by chromosome doubling. The manner of origin and even the mode of reproduction in the aneuploid species whose chromosome numbers approach $2n = 63$ cannot be deduced without a careful study of their meiotic and embryological processes.

II. CYTOLOGICAL VARIABILITY AND MODE OF SEED PRODUCTION IN *POA PRATENSIS* L.

The chromosome conditions in *P. pratensis* have been investigated and reported by several authors. Stahlin (14) published the number $2n = 56$. Avdulov (2) found the somatic numbers 28, 56 and 70. Nakajima (10) counted $2n = 70$. Müntzing (9) determined the chromosome numbers in eight biotypes, mostly of Swedish origin, and reported aneuploid chromosome numbers ranging from 64 ± 1 to 85 ± 1 . One heptaploid biotype ($2n = 49$) possessed that number in 10 individual plants examined. In preliminary studies on meiosis in this biotype the author observed the occurrence of irregularities which would lead to the formation of gametes with variable chromosome numbers. Rancken (11) examined four plants from a Finnish biotype and found the numbers to be aneuploid, varying between 66 and $67 + 2f$. In studies of reduction divisions in the P.M.C. this author also observed multivalent chromosome groups and lagging univalents. Müntzing drew the conclusion, with which Rancken is in agreement, that certain biotypes of *P. pratensis* form their seed apomictically. They offer as proof of apomixis the following conditions: (i) an aneuploid chromosome number which is constant for the biotype, (ii) the occurrence of irregularities at meiosis of the P.M.C., (iii) morphological constancy within the biotype, and (iv) good seed production.

Embryological investigations in *Poa pratensis* have been made by Anderson (1) and Nishimura (see (1)). Anderson observed the frequent occurrence of two embryo sacs within the same ovary. The author found the additional embryos to be formed from one of the three, usually nonfunctional, megaspores or from a separate megaspore mother cell. In no case did she observe additional embryos arising agamosporously. Nishimura found

that embryonic buds may arise from the antipodal nucellar region which may supplant the normal embryo. He regards these embryos of sporophytic origin as being the result of galls formed by insect attack.

In the present study plants from a wide range of material have been examined. This material consisted of (i) strains which have been subjected to selection and a fair degree of uniformity attained, (ii) indigenous plants from old sod in the Ottawa locality, and (iii) plants grown from commercial seed. The object of the study has been to ascertain the variability in chromosome number in plants from the various sources and to determine whether seed production is sexual or asexual.

DESCRIPTION OF MATERIAL

Ottawa No. 1. Selection in this strain has been carried on by the Division of Forage Plants during the past 15 years. It is a very uniform upright hay type which flowers earlier than any other selection (about May 20 at Ottawa). It is a good seed producer.

Aberystwyth No. 993. A very uniform pasture type, somewhat lacking in vigor and rather slow spreading. The leaves are waxy, giving the plants a distinct light green shade. It is comparatively late flowering and a fairly good seed producer.

Aberystwyth No. 994. A vigorous, spreading, narrow-leaved, pasture type. It is comparatively late flowering and a good seed producer.

Danish No. 939. A selection from a Danish pasture obtained from Dr. O. McConkey. While the strain as a whole is quite uniform it contains a small percentage of off-types. The predominant component is a uniform, wide-leaved pasture type, possessing fair vigor. It is medium-late flowering and the seed production is good.

Swedish No. 941. A uniform spreading pasture type obtained from Dr. O. McConkey. The leaves are intermediate in width and slightly waxy. It is late flowering and a good seed producer.

Mammoth No. 959. A selection from ordinary commercial material made at the Ontario Agricultural College. It is a uniform, vigorous, wide-leaved, pasture type with a tendency to become dormant in early summer. It is distinctly late flowering and a good seed producer.

Indigenous Plot. Plants taken at random from an old permanent pasture in the vicinity of Ottawa and propagated vegetatively. It is a mixture of pasture and hay types showing a wide variation in date of flowering, leaf width and general vigor.

Commercial (Local). Variable with regard to growth forms, pasture types predominating. It has a wide variation in morphological characters.

Commercial (Foreign). Variable with regard to growth forms but hay types predominating.

SOMATIC CHROMOSOME NUMBERS

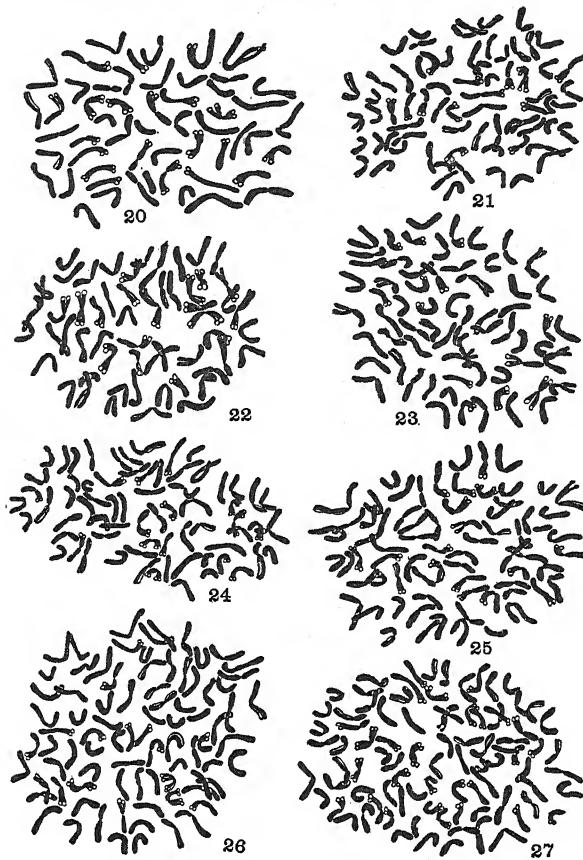
Table II gives the somatic chromosome numbers of the plants examined in the above lots. As a rule two plants were examined from each lot. In the case of Danish the plant with the lower chromosome number was an off-type. In most of the plants the chromosome number was ascertained with certainty but in certain plants, particularly the Aberystwyth, selections there may be an error of ± 1 .

TABLE II

LIST OF STRAINS OF *Poa pratensis* EXAMINED; SOURCE AND SOMATIC CHROMOSOME NUMBERS

Source	Accession number	No. of plants examined	Chromosome numbers
Danish pasture (O.A.C.)	939	2	50, 70 \pm 1
Swedish pasture (O.A.C.)	941	2	72, 72
Mammoth (O.A.C.)	959	2	54, 54
Aberystwyth	993	2	86 \pm 1, 87 \pm 1
Aberystwyth	994	2	84 \pm 1, 84 \pm 1
Ottawa selection	1	2	69 \pm 1, 70
Indigenous	—	2	64, 69 \pm 1
Commercial (Local)	—	2	50, 56
Commercial (Foreign)	—	3	56, 56, 65

Of the 19 plants examined, nine have euploid and ten have aneuploid chromosome numbers. All the euploids are even multiples of the basic number, 7, and are therefore capable of regular sexual reproduction. Of the six strains produced by selection, five possessed the same chromosome number in both plants examined. In the Danish strain, the plant typical of the strain had the euploid number 70 \pm 1 while the off-type plant had 50. The five plants examined from commercial material showed the euploid number ($2n = 56$) for three of the plants and the aneuploid numbers of 50 and 65 for the remaining plants. The two plants from indigenous material had a euploid number for one plant and an aneuploid number for the other. Typical plates of the various plants as examined are illustrated in Figs. 20–27. They all show about the same variability in chromosome length, with median or sub-median attachment constrictions. Swedish No. 941 (Fig. 6), with the aneuploid number $2n = 72$, possessed two chromosomes which are considerably shorter than the rest of complement, which suggests that they may be duplicate fragments. There is, therefore, a possibility of this biotype being derived from the euploid ($2n = 70$) by fragmentation.



FIGS. 20-27. Biotypes of *Poa pratensis* L. FIG. 20. Mammoth No. 959, $2n = 54$. FIG. 21. Danish No. 939, $2n = 70$. FIG. 22. Commercial (Local), $2n = 56$. FIG. 23. Indigenous, $2n = 64$. FIG. 24. Ottawa No. 1, $2n = 69 \pm 1$. FIG. 25. Swedish No. 941, $2n = 72$. FIG. 26. Aberystwyth No. 964, $2n = 83 \pm 1$. FIG. 27. Aberystwyth No. 993, $2n = 87 \pm 1$.

MICROSPOROGENESIS

In studying the reduction division in the pollen mother cells, smear preparations of the anthers were made using the method devised by McClintock (8). These smear preparations were supplemented with a few slides made by the paraffin method. Side views of the cell from heterotypic metaphase to homeotypic anaphase were examined for the occurrence of lagging univalents.

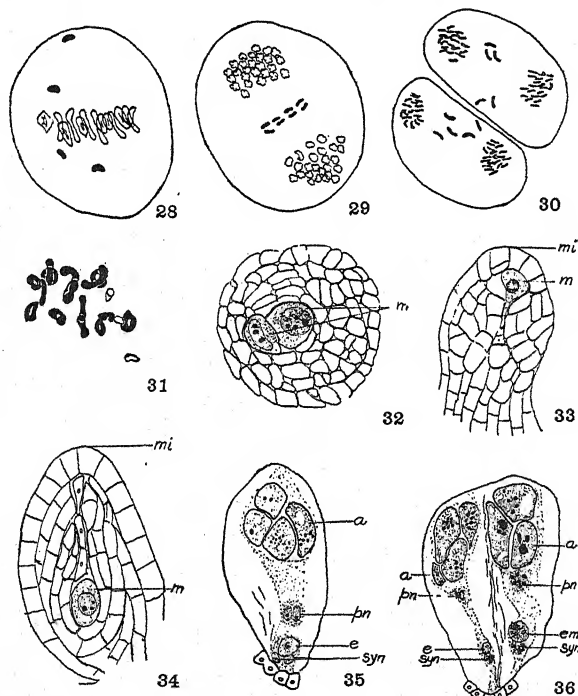
The results of this study are summarized in Table III.

TABLE III
FREQUENCIES OF POLLEN MOTHER CELLS WITH UNIVALENT CHROMOSOMES AND PERCENTAGE OF GOOD POLLEN IN STRAINS OF *P. pratensis*

—	No. of cells examined	Percentage of cells irregular	Av. no. of univalents per cell	Percentage of good pollen at dehiscence
Danish No. 839	43	2.3	.02	90.5
Swedish No. 941	25	8.0	.20	93.0
Aberystwyth No. 993	25	16.0	.28	87.0
Aberystwyth No. 994	21	32.0	.57	86.0
Mammoth No. 959	29	93.0	3.9	84.0

From the above table it may be seen that Mammoth was decidedly irregular in its meiotic behavior, the Aberystwyth strains slightly irregular, and the Danish and Swedish strains regular. Mammoth, with its very high percentage of irregular cells, afforded the best opportunity for observing the behavior of unpaired univalents. Their behavior was found to be typical of that described for the unpaired chromosomes in many interspecific hybrids (4). They divide equationally at the first division (Fig. 29), fail to divide at the second division and wander at random to either pole (Fig. 30). An examination of the pollen at the tetrad and later stages showed surprisingly few micronuclei, indicating that very few chromosomes fail to be included in the primary nuclei of the pollen grains.

In Table III the mean percentage of good pollen per strain is given. This was found to range from 84% for Mammoth to 93% for Swedish. As would be expected the percentages of normal pollen are negatively correlated with the percentages of irregularities at meiosis. One surprising fact is the relatively



FIGS. 28-31. Meiotic divisions in Mammoth (Not all the bivalents are drawn in any given cell). FIG. 28. First metaphase showing four univalents off the equatorial plate. FIG. 29. First telophase showing univalents dividing. FIG. 30. Second telophase showing univalents moving at random to poles. FIG. 31. First metaphase in E.M.C. showing the same type of bivalent configurations as in Fig. 28; three univalents are also present. FIG. 32. Two sister megaspores enlarging in the initial stage of polyembryony. FIG. 33. Lower megaspore nearest micropyle functional. FIG. 34. Upper megaspore most distant from micropyle functional. FIG. 35. Normal embryo sac just prior to fertilization. FIG. 36. Polyembryony after fertilization; only one egg has been fertilized. *a*, antipodals; *e*, egg; *em*, embryo; *m*, megaspore; *mi*, micropylar end; *pn*, polar nuclei; *syn*, synergids.

high percentage of good pollen in the Mammoth strain. Müntzing also found the pollen quite good in the *pratensis* biotypes which he examined, even the heptaploid biotype possessing 89% good pollen, in spite of considerable irregularity at meiosis.

POLLEN TUBE GROWTH

In the study of pollen germination and pollen tube growth, panicles of the plants in which many of the florets had shed their pollen a few hours previously were fixed and stored in 15% formalin. The pistils were then dissected out and mounted in lactic acid to which a few drops of aniline blue had been added.

Germination was found to be quite active, with considerable penetration of the pollen tubes into the stigmas and style. It was obviously impossible to make a statistical comparison of pollen germination for the various strains by the above method since most of the pollen that failed to germinate floated away in the mounting fluid. Each stigma had from 30 to 100 pollen grains actively germinated. Even the Mammoth strain, which showed a high proportion of irregularities at meiosis, showed good germination of the pollen. From this study it was concluded that the plants in all the strains examined produce abundant quantities of morphologically good pollen capable of germination.

EMBRYOLOGY

The observations on embryology covered the period from the prophase in the E.M.C. to post-fertilization, when the embryo had reached the several-celled stage. The observations were confined to plants from the selected strains, with the omission of Ottawa No. 1. Special emphasis was laid on Mammoth which showed the highest percentage of irregularities in the P.M.C. Daily fixations of the material were made with Navashin's and Bouin's fixatives. The whole floret with enclosed anthers and pistil was sectioned longitudinally at a thickness of 15μ and stained with gentian violet.

A generalized description of the course of embryo sac development applies to all the material examined. Departures will be noted later. The megaspore mother cell undergoes a heterotypic and homeotypic division to give rise to a row of four megaspores. A critical point in this study was to determine whether actual reduction in the chromosome number takes place. Pairing of chromosomes at the heterotypic metaphase was observed for all the strains as well as disjunction at anaphase. Observations of the homeotypic division were rarer but a few cells were observed at this stage. Fig. 31 illustrates a portion of a heterotypic metaphase in Mammoth. Not all the bivalents in the plate could be drawn, but a few bivalents typical of those observed in P.M.C. reduction were apparent. Three univalents could also be distinguished.

After the formation of the row of megaspores the one destined to function as the embryo sac gradually enlarges and the remaining three disintegrate. There did not seem to be any regularity as to the position of the functional

megaspore in the row, although it was more frequently the inner one farthest from the micropyle. Anderson (1) reported the same variability as to megaspore selection. Development of the female gametophyte proceeds normally, and just prior to fertilization there is present in the embryo sac a large egg and two smaller synergids at the micropylar end, two free polar nuclei in the protoplasmic strand connecting the egg and antipodals which lie at the chalazal end of the sac (Fig. 32). The antipodals are comparatively large and densely staining, varying from three to six in number.

The material which was fixed soon after anther dehiscence was examined carefully in the hope of observing the actual act of fertilization but in all observed cases the egg was either not yet fertilized or a two- to several-celled embryo was present. A significant observation was made in the strain Aberystwyth No. 994. A good polar view of a metaphase plate was obtained in a cell of the young embryo. A chromosome count that approximated the somatic number typical of the strain could readily be made. In the light of the evidence of reduction in the E.M.C. and good pollen germination this clearly points to the act of fertilization having taken place.

Polyembryony was found to be of common occurrence, although its frequency was higher in some strains than in others. This frequency was found to be 8, 11, 35, 42 and 42% for the strains 941, 939, 909, 993 and 984. It is significant that the amount of polyembryony occurring bears some relation to the frequency of univalents in the P.M.C. at meiosis. Thus, 941, which showed little irregularity at meiosis has a low frequency of polyembryony while Mammoth, which was quite irregular in its meiotic behavior has 35% polyembryony.

As to the origin of the two megaspores in the cases of polyembryony, Anderson found it difficult to determine whether the two embryo sacs arose from two separate E.M.C. or from two megaspores in a single row of megaspores, but inclines to the view that both methods of origin obtain. At the critical stage, when the method of origin can be determined, we have frequently observed two E.M.C. lying side by side in the prophase stage. As the P.M.C. in the accompanying anthers were also at diakinesis or first metaphase there could be little doubt that the cells in question were egg mother cells. However, in the majority of cases of polyembryony the enlarged cells appeared to be sister megaspores.

At later stages of development one embryo sac is usually crowded out by the other. In no case following fertilization have we observed two developing embryos, although this phenomenon has been reported by Anderson. A germination test was conducted on a seed sample of Mammoth but all the germinating seed showed single plumules, indicating that only one embryo per seed was functional.

DISCUSSION

Müntzing accounted for the occurrence of constant aneuploid chromosome numbers in certain biotypes of *P. pratensis* by assuming apomictical seed formation. In addition to cytological constancy he found that the different

biotypes were characterized by morphological constancy and good seed production. Preliminary studies on meiosis in a heptaploid biotype showed irregularities which he considered must lead to the formation of gametes with variable chromosome numbers. If these irregular gametes were functional the cytological constancy could not be preserved. Rancken found a similar situation in a Finnish biotype, but in this case the aneuploid chromosome number was not absolutely constant since it varied from 66 to 67 ± 2 ff. The evidence presented by the above authors unquestionably points to the operation of agamosporous seed formation but the present author feels that without some direct embryological evidence, the manner of reproduction in *P. pratensis*, whether sexual or asexual, cannot be definitely ascertained.

One of the strains, Mammoth No. 959, examined in the present study is similar to those examined by Müntzing and Rancken in every respect. It is uniform morphologically and a good seed producer. The two plants examined cytologically had the aneuploid chromosome number, $2n = 54$, and meiotic studies in the P.M.C. showed an average of 3.9 univalents per cell. Nevertheless embryological studies of this strain point to the mode of reproduction for this strain as being sexual.

Pollen germination and pollen tube growth were observed in all the strains. While this does not rule out agamosporous seed production, since pollen tube growth may be a required stimulus, it does meet one of the requirements of sexual reproduction.

Reduction in the E.M.C. was also observed in all the biotypes examined. This rules out the possibility of unreduced apogamy but leaves open the possibility of reduced apogamy—that is, the egg may fuse with another reduced cell in the female gametophyte, e.g., a synergid, and thus restore the diploid chromosome number. We are convinced that this is not a satisfactory explanation in the case of such a strain as Mammoth which has a low frequency of univalents in both P.M.C. and E.M.C. reduction. In such cases, if the functional megaspore did not contain the exact haploid chromosome complement, a fusion of two cells after the equational divisions in the female gametophyte could not restore the normal diploid number.

One of the significant results of the embryological studies noted was the frequent occurrence of polyembryony. The possibility of one of these embryo sacs arising aposporously from a cell of the nucellus or integument was considered. In the cases of apospory reviewed by Sharp (13) this aposporous budding out of cells from nucellar tissue takes place after the normal gametophyte has reached the eight-celled stage and results in an undifferentiated group of cells, which replaces the normal gametophyte. In the present studies the twin embryos were observed to arise simultaneously and both contained the differentiated cells, egg, synergids, polar and antipodals. Hence the double embryos present in many ovaries have in all probability a common origin.

A hypothesis will now be advanced in an attempt to explain how a strain like Mammoth which apparently is reproduced sexually, may maintain a constant aneuploid chromosome number.

In the study of microsporogenesis in Mammoth an average number of four unpaired univalents per cell was observed. At the tetrad stage and later the absence of micronuclei was also noted and this indicates that the lagging univalents have been included in the primary nuclei in the majority of cases. If the assortment of these four univalents were at random in the homeotypic division the theoretical frequency of pollen grains containing 0, 1, 2, 3, and 4 univalents (in addition to the fixed number 25) would be 1, 4, 6, 4 and 1 respectively. On this basis 6/16 of the pollen grains would contain the normal haploid number of 27 chromosomes. On this hypothesis it would be necessary to assume that only pollen grains with the normal chromosome complement function and this assumption is in agreement with results obtained in wheat species crosses (15). Considering the high chromosome number of Mammoth and other *pratensis* biotypes it is unlikely that the genetic balance would be disturbed by the random segregation of the univalents. If it were disturbed, and genetic balance as well as constant chromosome number were essential, then the production of viable pollen grains would be much reduced.

In the embryological studies a variation in the position of the functioning megaspore in the row of four megaspores was noted. This, we believe, provides a mechanism for the elimination of megaspores with an abnormal chromosome complement and for the choice of the megaspore containing the normal chromosome complement. Assuming the same frequency of irregularity in megasporogenesis as in microsporogenesis, 6/16 of the megaspores would be normal, and with a choice of four megaspores the chance of obtaining one with the normal number is quite good. As previously shown the frequency of polyembryony in the different strains is related to the degree of irregularity of meiosis. Strains 939 and 941, which are almost completely regular at meiosis, have a low frequency while Mammoth has a high frequency of polyembryony associated with its irregular meiotic behavior.

The phenomenon of polyembryony, both in the development of two megaspores and the later elimination of the additional embryo sac, may be regarded as evidence that there is a selective tendency in certain strains of *P. pratensis* towards a choice of normal megaspores. Polyembryony appears to be a response to meet the situation of irregular chromosome behavior.

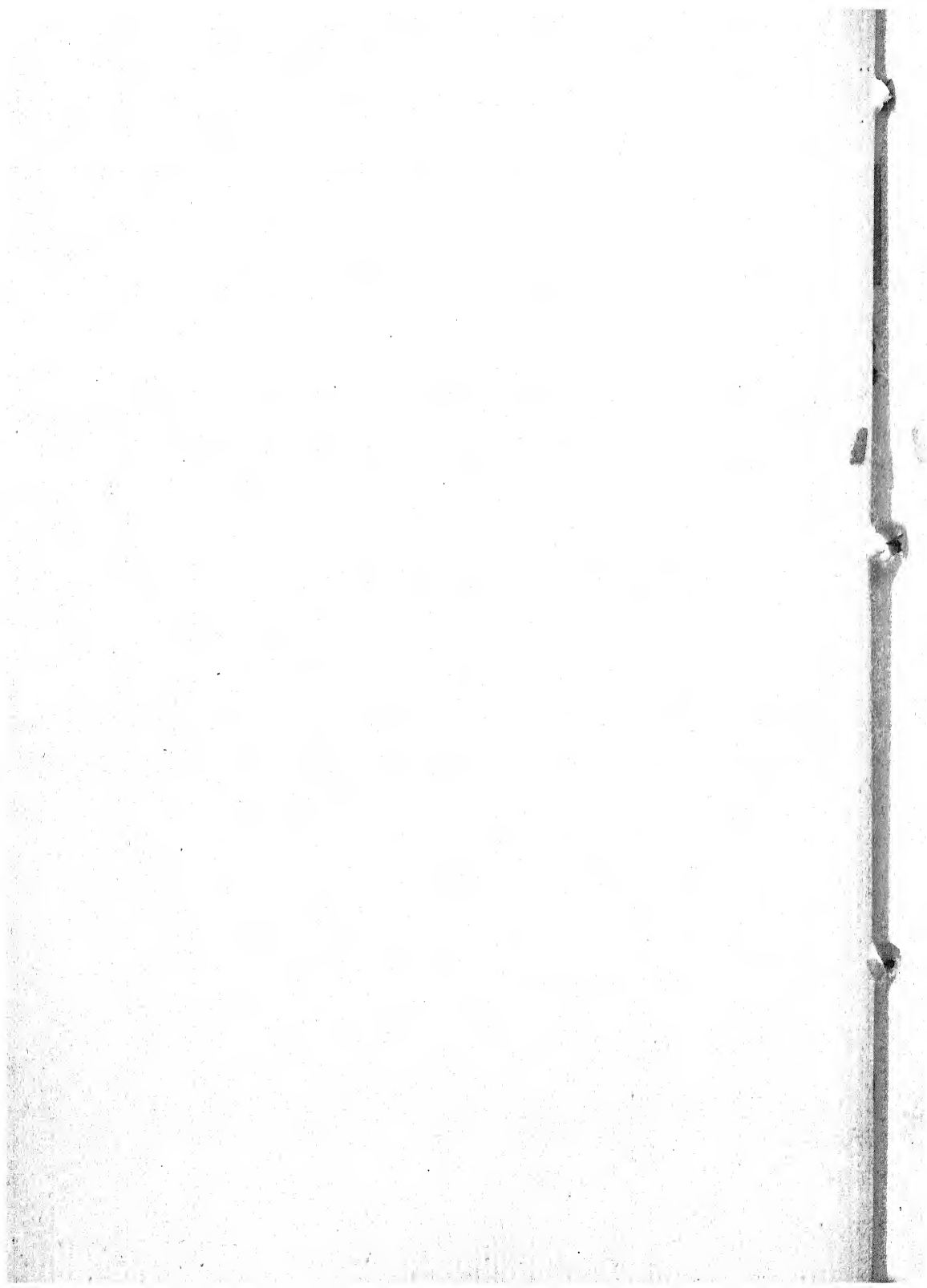
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CHEMICAL WEED KILLERS

I. RELATIVE TOXICITY OF VARIOUS CHEMICALS TO FOUR ANNUAL WEEDS¹

By W. H. COOK²

Abstract

Toxicity tests on four annual weeds, *Thlaspi arvense* L.; *Brassica arvensis* L. Ktze.; *Chenopodium album* L.; and *Avena fatua* L.; showed no definite evidence of a specific susceptibility of a given species to a given substance. The relative resistance of these four weeds to most substances, judging from the certainly lethal dose, was in the order 1 : 1 : 2 : 7. Of the 76 chemicals tested, the following most toxic compounds killed all four species at the dosages employed; selenic and chloric acids, sodium hydroxide, arsenic pentoxide, sodium arsenite, sodium and ammonium chlorate, ammonium thiocyanate, sodium cyanide, zinc chloride, sodium bichromate, sodium selenite, copper nitrate, sodium sulphide, formic acid, gasoline, phenol, creosote, tetralin, sodium benzoate, aniline, benzene and furfural. The residual toxic effect on the soil, three to four weeks after treatment, showed that of the 35 more toxic chemicals tested, only selenic acid and the five chlorates used had any appreciable effect at low and intermediate dosages, while eleven other substances depressed growth following the application of high dosages.

Introduction

This investigation was undertaken to determine the relative toxicity of a number of selected chemicals to four annual weeds. Similar experimental conditions and methods, and the same criterion of toxicity, were used throughout. In reviewing the literature (4) it became evident that, although a great many experiments had been made on the toxicity of chemicals to plants, the comparable data were so few that it was impossible to estimate the relative toxicity of different substances. The variable results reported by different investigators are doubtless due partly to the different species used, and to the various growth conditions under which the determinations were made. However, much of the variability can be attributed to the methods of application and to the criteria of efficacy employed by different workers.

Annual weeds were used in these experiments, as they grow rapidly from seed and also permit the efficacy of the treatment to be estimated from the condition of the leaves and stems. Mortality was selected as the criterion of the efficacy of the treatment, since it is the one generally used in analogous studies, and more is known about the relation between dosage and mortality, than between dosage and the reduction of weight or growth rate, or other physiological effects. The effect on plant size may of course be important

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under field conditions. Most of the accurate work on dosage-mortality relations has been done with animals, where the chemical can be administered directly into the individual, or where a number of individuals can be subjected to a known concentration, as in fumigation in closed spaces. Under these conditions the dosage-mortality curve is usually sigmoid, representing an integrated frequency curve descriptive of the variable susceptibility of apparently similar individuals of a population. Since these curves usually have their maximum slope in the region of 50% mortality, Trevan (6) has pointed out that the relative toxicity of different substances can be determined with the least error if the dosages administered produce mortalities falling within the relatively linear portion of the curve, *i.e.*, between about 25 and 75% mortality. More recently Bliss (1, 2) has described methods for transforming the dosage-mortality curve to a straight line, thus permitting all of the available data to be used for estimating the toxic properties of a compound.

When this investigation was begun in 1930, it was found that the data available in the literature were wholly inadequate for estimating dosages that would produce mortalities between about 25 and 75%. Furthermore, preliminary determinations of the shape of the dosage-mortality curve for some of the chemicals to be tested indicated that several did not yield a sigmoid curve. This might be due to a decided asymmetry in the distribution of the susceptibilities of the individuals; but the method of experiment, in particular the application of the chemical as a spray rather than administration into the organism, seemed a more likely explanation, since an unknown proportion of the spray drains to the soil where it may be rendered ineffective. This phase of the work will be discussed in greater detail in a later paper.

These preliminary experiments led to the use of the certainly lethal dose (C.L.D.) as an index of toxicity. The form of some of the dosage-mortality curves indicated that this quantity could be determined about as accurately as the median lethal dose. In any event, the chemicals under investigation varied so widely in toxicity that the C.L.D. permitted their initial classification. The number of tests to be made was also reduced by limiting the dosage range. Substances which did not produce a useful mortality at the highest dosage tried were considered useless as herbicides, while those that were effective at the lowest dosage merited further investigation.

Materials and Methods

The following weeds were used: stinkweed, *Thlaspi arvense* L.; wild mustard, *Brassica arvensis* (L.) Ktze.; lamb's quarters, *Chenopodium album* L.; and wild oats, *Avena fatua* L. These species represent the four most troublesome annuals in western Canada, and include some diversity in leaf form, growth habit, and resistance to chemicals.

The plants were grown from seed planted directly in the experimental crocks. Seeds in excess of the number of plants required were sown, and later thinned out to five plants per crock, excepting wild oats, of which 10

plants per crock were used. The soil was composed of two parts surface loam and one part sand; its moisture content was held at the optimum for growth (taken as half the moisture holding capacity). Other growth conditions were not controlled, but the mean daily temperature usually fell within the range 65° to 70° F., and the relative humidity 45% to 65%. During the autumn, winter and spring season, the natural period of daylight was supplemented by using a 300 watt Mazda lamp over each 15 sq. ft. of bench space, from sunset until midnight.

The period between seeding and treatment varied from three to four weeks. An attempt was made to obtain plants at the same stage of development rather than of the same age. In three to four weeks, the stinkweed was in the rosette stage, 3 to 4 in. high and had from 15 to 25 leaves per plant; the wild mustard was 6 to 8 in. tall, and had from 6 to 10 leaves per plant; the lamb's quarters was also from 6 to 8 in. tall and had from 18 to 30 leaves per plant; and the wild oats was 9 to 12 in. tall and was in the 3- to 4-leaf stage. The crocks for a given weed were seeded in groups of 60, using the same soil, seed, etc., and were all sprayed on the same day. Ten crocks in each group were used as treated and untreated controls. The treated controls were sprayed with sulphuric acid or sodium chlorate, the dosages used being in the region of the C.L.D. These sprayed controls revealed groups of plants abnormally resistant or susceptible to chemical treatment. The untreated controls were used as a basis for computing, from the size and weight of the plants, the efficacy of treatments which did not give complete mortality.

The different chemicals varied greatly in their rate of action on the plants. Some produced 100% mortality within a day or two, while others had no evident effect during several days, after which the mortality increased steadily with time. Obviously a time limit had to be set on the observations. In the initial experiments, the mortality increased very slowly, if at all, after three to four weeks, and this period was chosen as the time limit. It cannot be said, however, that a higher mortality would not have been observed had the plants treated with sub-lethal doses of these chemicals been allowed to remain under observation for a longer time.

Most of the 76 chemicals tested had already been used as herbicides, but their relative toxicity had never been investigated under comparable conditions. Some were included because the literature indicated that they had a toxic effect on plants when used as a spray for controlling insects, fungi, etc. A few substances related to compounds known to be toxic were also included, as well as some substances of low solubility in water, and some of relatively high volatility. All of the chemicals sufficiently soluble in water were sprayed as a 10% solution, a few substances of lower solubility as a 5% solution, and insoluble solids were applied as a dust. The liquid substances that were immiscible with water were usually applied directly. Where small doses of undiluted liquids were applied, it is probable that their apparent toxicity was somewhat too low, since it was impossible to cover the plants adequately with such small volumes.

The substances were applied at the following dosages:— 0.3, 0.5, 0.75, 1.00, 1.50, 2.00 and 2.5 gm. per crock, which in terms of surface area of soil corresponded to amounts of about 125, 210, 320, 420, 620, 840, and 1050 lb. per acre. Since most investigators are accustomed to think in terms of rates per acre, the results have been tabulated on this basis. Wild oats were found to be much more resistant to chemicals than the other weeds, and a dosage of 5.00 gm. per crock, or 2100 lb. per acre was also employed on this weed. These quantities are the actual amount of chemical added and not the total amount of liquid sprayed when application was made as a solution. No additional information would have been secured had the dosage increments been smaller, owing to the magnitude of the variability and experimental error encountered in such tests as these. Each determination consisted of applying 3 to 5 adjacent dosages, within the range of the expected C.L.D., to duplicate crocks of plants. The lowest dosage giving complete mortality was taken as the C.L.D. and the test was repeated if the results were not consistent with those obtained at higher and lower dosages.

The spraying equipment consisted of an Atlas No. 29 atomizer nozzle attached to a vessel of suitable size to accommodate the amount of solution used. Compressed air under a pressure of about 5 lb. per sq. in. gave a continuous spray. Several types of dusting nozzles were designed and tested before a suitable one was obtained for applying the insoluble solids. The main difficulty was to get one that would transfer quantitatively the small amounts of the finely ground adherent dusts to the plant, owing to their tendency to adhere to the duster. This difficulty was overcome by providing an auxiliary air jet in the dust chamber arranged to cause a swirling movement of the material, which had an abrasive action that dislodged adherent particles. Before spraying or dusting, a cylindrical sheet-metal guard, lined with wax paper, was placed around the plants, to minimize the loss of finely divided material. The nozzle was placed just over this guard, pointing downward across the plants at an angle of about 45°. In order to expose all parts of the plant, as would happen with moving apparatus in the field, the crocks were rotated, by means of a turntable, at about 50 revolutions per minute while being treated.

Results

EXPERIMENTAL ERROR AND SEASONAL VARIABILITY

Before considering the results of the main series of tests it is necessary to form some estimate of the experimental error. The data, in most cases, were inadequate for making precise estimates of the variability, and since a sigmoid relation between dosage and mortality was obtained in relatively few instances, the methods of Trevan (6) and Bliss (1, 2) could not be applied directly. During the course of the experiments it was found that duplicate crocks of plants from the same lot, treated at the same time, seldom differed significantly. If a given dosage killed all of the plants in one crock, a similar result was usually obtained in the others, but in a few cases only an 80% kill would be observed. Instances of greater variability were rare. When a complete kill was not obtained in duplicate crocks the test was repeated.

Since repetitions had to be made on a different lot of plants, it soon became evident that the variability in the results between lots exceeded that within lots grown at the same time. Sometimes a chemical would give only a partial mortality at one dosage on one lot of plants, while on another it would give a complete kill, and *vice versa*, although both lots appeared to be about equally resistant to control treatments of sodium chlorate and sulphuric acid. An anomalous relation was observed in only a few instances between a given dosage and the second higher dosage. Since the ratio of one dose to the second higher dose was about two, it appears that in order to be considered significant, the reported C.L.D.'s of the chemicals to one species must differ from each other by at least 100%. Since the maximum variability described above was confined almost entirely to the higher dosages, it seems likely that the results obtained with the more toxic chemicals are subject to less error.

The above discussion applies to results obtained on successive lots of a given weed treated on different weeks during the same season, where the resistance of the plants appeared to increase or decrease from lot to lot more or less at random. At the outset it was planned to carry out these tests throughout the entire year but it was found that, in addition to the random variability, there was a systematic variability from season to season, winter-grown plants being more susceptible to chemical treatment than summer-grown plants. The C.L.D. of several chemicals to both summer- and winter-grown plants are given in Table I. It is evident that most of the chemicals kill winter-grown plants at about one-half the dosage required in the summer. Sodium chlorate is an exception, the quantity required for a complete kill being about

TABLE I
SEASONAL VARIATION IN RESISTANCE OF PLANTS TO CHEMICALS

Chemical	Weed	Winter, October to March inclusive, (C.L.D.), lb. per acre	Summer, April to September inclusive, (C.L.D.), lb. per acre
Sulphuric acid	Stinkweed	—	210
Sulphuric acid	Wild mustard	125	320
Sulphuric acid	Lamb's quarters	210	420
Sulphuric acid	Wild oats	1050	>2100
Sodium chlorate	Stinkweed	125-320	125-320
Sodium chlorate	Wild mustard	210-320	210-320
Sodium chlorate	Lamb's quarters	320-420	420-525
Sodium chlorate	Wild oats	1650	1650-1860
Zinc chloride	Stinkweed	125	125-210
Zinc chloride	Wild mustard	210	320
Ammonium sulphate	Wild mustard	420	840
Nickel sulphate	Wild mustard	620	1050
Selenic acid	Wild mustard	125	210
Sodium bichromate	Lamb's quarters	125	320
Zinc sulphate	Lamb's quarters	620	1050-1260
Copper nitrate	Wild oats	840	2100
Sodium hydroxide	Wild oats	840	1650

the same in both seasons. This might be due to the slow poisoning action observed with this substance, which necessitated the use of relatively high dosages in order to produce the required mortality within the 3- to 4-week observation period employed. The greater susceptibility of winter-grown plants to most of the chemicals indicated that comparative data could only be obtained during the same growth season. All subsequent tests reported in later tables were obtained during the summer, *i.e.*, April to September inclusive. The results in Table I, however, indicate one cause of the seasonal variability in the efficacy of herbicides applied in the field.

RELATIVE SUSCEPTIBILITY OF SPECIES AND TOXICITY OF CHEMICALS

In presenting the results of the main series of experiments, the various chemicals have been classified into the following groups: acids and alkalis; arsenicals; chlorates and related compounds; cyanides and related compounds; halides, sulphates and related compounds; miscellaneous inorganic compounds; aliphatic inorganic compounds, aromatic organic compounds; and industrial by-products. The data obtained are given in Tables II to XI inclusive. The individual chemicals in each group are arranged from top to bottom in the approximate order of decreasing toxicity, as judged from the results obtained with all four weeds. Where a considerable variation in toxicity occurred within a single group of chemicals, the certainly lethal dose (C.L.D.) and the time required for complete mortality is reported for the more toxic chemicals, and the percentage mortality at the maximum dosage applied for the others.

In these tables, the results for each of the four weeds appear in the order, stinkweed, wild mustard, lamb's quarters and wild oats. Examination of the data shows that, for the majority of the chemicals, this is in order of increasing resistance. Where the order of relative toxicity of the chemicals to one species differs, beyond the estimated experimental error, from that obtained on the others, a specific susceptibility of that particular species to particular substances may be suggested. This happened so infrequently that, until more precise measurements are made with more plant species, they can more safely be attributed to the variability inherent in experiments of this type. Furthermore, it would appear that, in field practice, a herbicide having a high specific toxicity to one or two species, but a low toxicity to the others, would be of little value for general use.

The relative resistance of the four species was compared by plotting the C.L.D.'s of the chemicals for stinkweed, wild mustard and wild oats against the C.L.D. of the same chemical for lamb's quarters, which has an intermediate resistance. The resulting graph is shown in Fig. 1. Of the 76 chemicals tested, only about 23 caused complete mortality to all four weeds at the dosages used and some of these were only completely lethal at the highest dosage employed. Since there is a large discrepancy between the "applied" and "retained" quantities with the large volumes of spray required to apply the maximum dosages used in these experiments, these high doses were also

TABLE II
TOXICITY OF INORGANIC ACIDS AND ALKALI TO FOUR ANNUAL WEEDS

Chemical	Method of application	Stinkweed		Wild mustard		Lamb's quarters		Wild oats	
		C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days
Selenic acid	Spray 10% soln.	125	1	210	—	210	18	620	4
Chloric acid	Spray 10% soln.	125	1	125	18	210	18	1050	22
Sodium hydroxide	Spray 10% soln.	125-210	1 to 2	210	20	320	21	1650	21
		Dosages below C.L.D.							
								Dose, lb. per acre	Mortality, %
Hydrofluosilicic acid	Spray 10% soln.	125	2	160	5	320	4	1050	62
Hydrochloric acid	Spray 10% soln.	125	1	125	18	320	4	2100	84
Nitric acid	Spray 10% soln.	125	1	320	—	320	21	1650	64
Sulphuric acid	Spray 10% soln.	210	2	320	1 to 6	420	4	2100	46

TABLE IV
TOXICITY OF CHLORATES AND RELATED COMPOUNDS TO FOUR ANNUAL WEEDS

Chemical	Method of application	Stinkweed		Wild mustard		Lamb's quarters		Wild oats	
		C.L.D., lb. per acre	Time required for com- plete kill, days	C.L.D., lb. per acre	Time required for com- plete kill, days	C.L.D., lb. per acre	Time required for com- plete kill, days	C.L.D., lb. per acre	Time required for com- plete kill, days
Sodium chlorate Ammonium chlorate Barium chlorate	Spray 10% soln.	125-320	4 to 26	210-320	18 to 23	420-525	18	1650-1860	21
	Spray 10% soln.	(320)	4	210	17 to 21	(840)	—	1650	22
	Spray 10% soln.	320	21	210	21	525	50% mortality	2100	22
Calcium chlorate	Spray 10% soln.	210	22	210	20	525	18	Dosages below C.L.D.	
								Dose, lb. per acre	Mortality, %
Calcium hypochlorite Sodium perchlorate	Spray 10% soln. (Some suspend- ed material) Spray 10% soln.	420 840	23	620	4	1050	90	2100	34
			22	840	17		54	2100	72

Bracketed values doubtful; obtained by interpolation. Value in bold-faced type in C.L.D. column indicates applied dosage, not C.L.D.

TABLE V
TOXICITY OF CYANIDES AND RELATED COMPOUNDS TO FOUR ANNUAL WEEDS

Chemical	Method of application	Stinkweed		Wild mustard		Lamb's quarters		Wild oats	
		C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days
Ammonium thiocyanate Sodium cyanide	Spray 10% soln.	125	3	125	21	125	22	410	20
	Spray 10% soln.	125	4	125	6	210	4	525	22
Dosages below C.L.D.									
Sodium ferrocyanide	Spray 10% soln.	420	25	210	21	1050	17	2100	79
Dosages below C.L.D.									
Calcium cyanamide	Dust	Dose, lb. per acre	Mortality, %	Dose, lb. per acre	Mortality, %	Dose, lb. per acre	Mortality, %	—	—
		1050	0	460	20	1050	50		

TABLE VI

TOXICITY OF HALIDES TO FOUR ANNUAL WEEDS

Chemical	Method of application	Stinkweed		Wild mustard		Lamb's quarters		Wild oats	
		C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days	Dose, lb. per acre	Mortality, %
Zinc chloride	Spray 10% soln.	125-210	2 to 14	320	21	420	18	2100	94
		620	23	620	21	Dosages below C.L.D.		2100	0
Calcium chloride Sodium chloride	Spray 10% soln. Spray 10% soln.	—	—	—	—	1050	16	—	—
		—	—	Dosages below C.L.D.		2100	40	2100	0
Potassium chloride	Spray 10% soln.	—	—	2100	40	2100	40	2100	0
Cryolite	Dust	Dosages below C.L.D.		420	0	1050	0	—	—
		Dose, lb. per acre	Mortality, %	1050	0	—	—	—	—

TABLE VII
TOXICITY OF SULPHATES AND RELATED COMPOUNDS TO FOUR ANNUAL WEEDS

Chemical	Method of application	Stinkweed		Wild mustard		Lamb's quarters		Wild oats	
		C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days	Dosages below C.L.D. Dose, lb. per acre	Mortality, %
Zinc sulphate	Spray 10% soln.	840	14	1050	21	1050-1260	17	2100	5
						Dosages below C.L.D.			
						Dose, lb. per acre	Mortality, %		
Copper sulphate	Spray 10% soln.	125	4	125	20	1050	85	2100	38
Ammonium sulphate	Spray 10% soln.	1050	14	840	21	—	—	2100	0
Ferrous sulphate	Spray 10% soln.	1050	3	1050	80% mortality	1050	90	2100	0
		Dosages below C.L.D.							
		Dose, lb. per acre	Mortality, %						
Sodium sulphite	Spray 10% soln.	1050	40	1050	4	—	—	2100	0
Nickel sulphate	Spray 10% soln.	1050	70	1050	20	1050	0	2100	0

TABLE VII—*Concluded*
TOXICITY OF SULPHATES AND RELATED COMPOUNDS TO FOUR ANNUAL WEEDS—*Concluded*

Chemical	Method of application	Stinkweed		Wild mustard		Lamb's quarters		Wild oats	
		C.L.D., lb. per acre	Time required for com- plete kill, days	C.L.D., lb. per acre	Time required for com- plete kill, days	C.L.D., lb. per acre	Time required for com- plete kill, days	Dosage below C.L.D. lb. per acre	Mortality, %
				Dosages below C.L.D.					
				Dose, lb. per acre	Mortality, %				
Ferric sulphate	Spray 10% soln.	1050	78	1050	88	—	—	2100	40
Aluminium sulphate	Spray 10% soln.	1050	0	1050	10	1050	12	2100	10
Chromium amm. sulphate (alum)		840	0	1050	10	1050	0	2100	0
Nickel ammonium sulphate (alum)	Spray, sat. soln. (10% approx.)	1050	80	—	—	1050	0	2100	0
Aluminium amm. sulphate (alum)	Spray 10% soln.	1050	0	1050	0	1050	0	2100	0
Aluminium pot. sulphate (alum)	Spray 10% soln.	1050	0	1050	0	1050	0	2100	0

Value in bold-faced type in C.L.D. column indicates applied dosage, not C.L.D.

TABLE VIII
TOXICITY OF MISCELLANEOUS INORGANIC COMPOUNDS TO FOUR ANNUAL WEEDS

Chemical	Method of application	Stinkweed		Wild mustard		Lamb's quarters		Wild oats	
		C.L.D., lb. per acre	Time required for com- plete kill, days	C.L.D., lb. per acre	Time required for com- plete kill, days	C.L.D., lb. per acre	Time required for com- plete kill, days	C.L.D., lb. per acre	Time required for com- plete kill, days
Sodium dichromate Sodium selenite Copper nitrate Sodium sulphide	Spray 10% soln.	125	5	125	17	320	4	525	22
	Spray 10% soln.	125	3	125	21	210	17	840	4 to 21
	Spray 10% soln.	(125)	—	125	20	320	18	2100	4
	Spray 10% soln.	320	14	620	2	320	21	2100	
Sodium carbonate	Spray 10% soln.	420	23	840	21	1050	10	2100	No mortality
				Dosages below C.L.D.		Dosages below C.L.D.			
Carbon disulphide	Undiluted liquid injected into soil	2700	22	2700	50	2700	50	5400	19
								Dosages below C.L.D.	
		Dose, lb. per acre	Mortality, %	Dose, lb. per acre	Mortality, %	Dose, lb. per acre	Mortality, %	Dose, lb. per acre	Mortality, %
		1050	90	—	—	1050	0	1050	0
Ammonium phosphate Sodium silicate Potassium permanganate Lead nitrate Sodium tetraborate	Spray 10% soln.	1050	50	1050	0	1050	0	2100	0
	Dust	1050	0	1050	0	1050	0	740	0
	Spray sat. soln.	370	—	1050	0	1050	0	1050	0
	Spray 10% soln.	—	—	1050	20	—	—	—	—

Bracketed values doubtful; obtained by interpolation.

Value in bold-faced type in C.L.D. column indicates applied dosage, not C.L.D.

TABLE IX
TOXICITY OF ALIPHATIC ORGANIC COMPOUNDS TO FOUR ANNUAL WEEDS

Chemical	Method of application	Stinkweed		Wild mustard		Lamb's quarters		Wild oats	
		C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days
Formic acid Formamide Gasoline (ethyl) Sodium acetate Kerosene	Spray 10% soln.	210	3	125	1	125	3	1050	4
	Spray 10% soln.	420	2	620	20	1050	4	2100	72% mortality
	Spray undiluted	940	6	1550	5	940	3	1550	2 to 10
	Spray 10% soln.	840	17	1050	4	1050	No mortality	2100	No mortality
	Spray undiluted	—	—	6850	4	—	—	5350	4
		Dosages below C.L.D.							
Ethylene dichloride Methanol		Dose, lb. per acre	Mortality, %						
	Spray undiluted	1320	84	1320	90% mortality	3950	4	5250	4
	Spray 95% soln.	1650	0	16800	17	—	—	—	—
		Dosages below C.L.D.							
Acetone Ethyl acetate Urea Motor oil		Dose, lb. per acre	Mortality, %	Dose, lb. per acre	Mortality, %	Dose, lb. per acre	Mortality, %	Dose, lb. per acre	Mortality, %
	Spray 10% soln.	1050	0	1050	0	1050	0	2100	0
	Spray 10% soln.	1050	0	1050	0	1050	0	2100	0
	Spray 20% soln.	—	—	2100	50	2100	0	2100	0
	Spray undiluted	3860	0	4800	88	—	—	—	—

Values in bold-faced type in C.L.D. column indicate applied dosages, not C.L.D.

TABLE X—*Concluded*
TOXICITY OF AROMATIC (CYCLIC) ORGANIC COMPOUNDS TO FOUR ANNUAL WEEDS—*Concluded*

Chemical	Method of application	Stinkweed		Wild mustard		Lamb's quarters		Wild oats	
		C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days
Naphthalene Sodium naphthionate	Dust Spray 10% soln.	1050 1050	5 17 to 22	Dosages below C.L.D.		1050 —	0 —	1050 1050	5 0
				Dose, lb. per acre	Mortality, %				
		1050 1050	0 0	1050 460	10 10	1050 1050	25 0	2100 —	5 —
				Dosages below C.L.D.					
Pyridine Naphthionic acid	Spray undiluted Dust	1050 1050	0 0	Dosages below C.L.D.					
				Dose, lb. per acre	Mortality, %				

TABLE XI
TOXICITY OF INDUSTRIAL BY-PRODUCTS TO FOUR ANNUAL WEEDS

Chemical	Method of application	Stinkweed		Wild mustard		Lamb's quarters		Wild oats	
		C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days	C.L.D., lb. per acre	Time required for complete kill, days	Dosages below C.L.D. Dose, lb. per acre	Mortality, %
Tar acids	Spray 10% emulsion	125	2	210	21	210	7	1470	89
		Dosages below C.L.D.						2100	0
Sulphite liquor	Spray undiluted	—	—	Dose, lb. per acre	Mortality, %	Dose, lb. per acre	Mortality, %		
		—	—	2100	0	2100	0	2100	0

excluded from the comparisons. A dotted line represents the results obtained with wild oats where the position of the curve is less certain owing to the small number of data available. In all cases straight lines were fitted to the points graphically. It should be noted that since extrapolations of these lines would not pass through zero on both axes, the relation over a longer range might be represented by a curve of a second or higher order. Fig. 1 shows definitely that a chemical which is more toxic than another to one weed will in general be the more toxic to other species. The relative resistance of the four species used cannot be determined with precision from the available data, but by choosing a substance having an intermediate C.L.D. of 400 lb. per acre for lamb's quarters, it can be seen from the figure that about 200 lb. per acre will be the C.L.D. for stinkweed and wild mustard. A chemical that will kill lamb's quarters at 200 lb. per acre will have to be applied at a rate of about 700 lb. per acre to kill wild oats. From these quantities, the approximate relative resistance of the four species to chemicals is: stinkweed : wild mustard : lamb's quarters : wild oats :: 1 : 1 : 2 : 7.

The chemicals used were divided into three main classes, on the basis of the number of species killed at the dosages employed, namely: those which gave complete mortality on all four species; those which gave complete mortality on only two or three species; and those which did not give complete mortality on any, or more than one, species. Obviously these main classes can be subdivided further if the difference between them exceeds the estimated experimental error.

Since an increase in dosage of about 100% was estimated to cover the experimental error, the subdivision of the first class, which killed all of the plants within the dosage range used, into three groups (I, II, and III) appeared to be justified. This subdivision was made on the basis of the number of species killed within the minimum, intermediate and maximum dosage ranges as defined in Table XII where the chemicals are classified. It is also possible to group chemicals of the second class according to whether they killed two

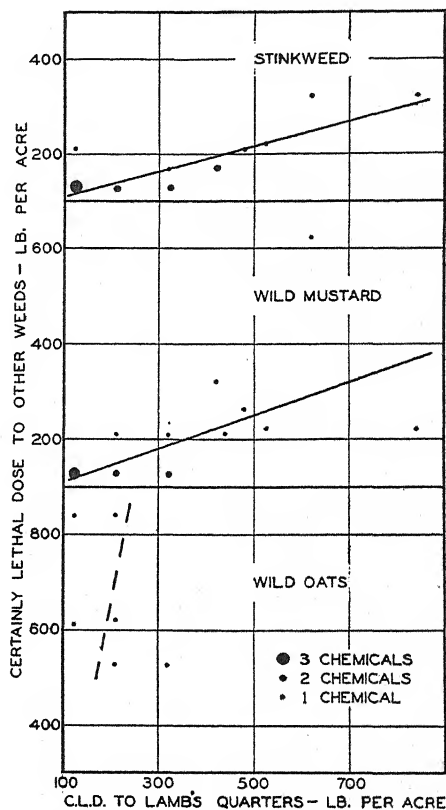


FIG. 1. Resistance of stinkweed, wild mustard and wild oats relative to lamb's quarters.

TABLE XII

CLASSIFICATION OF CHEMICALS IN ACCORDANCE WITH THEIR RELATIVE TOXICITY

(Read left to right across pages 318-319)

Group No.	Acids and alkali (Table II)	Arsenicals (Table III)	Chlorates, etc. (Table IV)	Cyanides, etc. (Table V)
I. Complete mortality on all four weeds, C.L.D. in min. dosage range for at least three weeds and not exceeding intermediate dosage range for fourth.	Selenic	Arsenic pentoxide		Ammonium thiocyanate, Sodium cyanide
II. Complete mortality on all four weeds, C.L.D. for at least one weed in min. dosage range, and for not more than one, in max. dosage range.	Chloric, Sodium hydroxide	Sodium arsenite	Sodium chlorate, Ammonium chlorate	
III. Complete mortality on all four weeds, C.L.D. at, or below, max. dosages employed, but not meeting requirements for Groups I and II.				
IV. Complete mortality on three weeds. Sum of percentage mortalities 350 or over.	(Hydrofluosilicic), Hydrochloric, Nitric	Sodium arsenate	(Barium chlorate), Calcium chlorate	Sodium ferrocyanide
V. Sum of percentage mortalities 300 or over.	Sulphuric		Calcium hypochlorite, Sodium perchlorate	
VI. Sum of percentage mortalities 200 or over.				
VII. Sum of percentage mortalities 100 or over.				
VIII. Sum of percentage mortalities less than 100.		Lead arsenate		(Calcium cyanamide)

Minimum dosage range = 125-320 lb. per acre. Intermediate dosage range = 420 to 840 lb. per acre. Maximum dosage range—1050 lb. per acre on dicotyledonous weeds, 1050 to 2100 lb. per acre on wild oats.

Position of bracketed chemicals doubtful.

TABLE XII

CLASSIFICATION OF CHEMICALS IN ACCORDANCE WITH THEIR RELATIVE TOXICITY

(Read left to right across pages 318-319)

Halides (Table VI)	Sulphates, etc. (Table VII)	Misc. inorganic compounds (Table VIII)	Aliphatic organic compounds (Table IX)	Aromatic organic compounds (Table X)	Industrial by-products (Table XI)
		Sodium bichromate, Sodium selenite		Phenol	
Zinc chloride		Copper nitrate	Formic acid	(Creosote)	
		Sodium sulphide	(Gasoline)	Tetralin, Sodium benzoate, Aniline, (Benzene), Furfural	
				Sodium salicylate	Tar acids
	Zinc sulphate, Copper sulphate		Formamide	α -Naphthy- lamine	
(Calcium chloride), (Sodium chloride)	(Ammonium sulphate), (Ferrous sulphate)	Sodium carbonate, (Carbon disulphide)	Sodium acetate, (Ethylene dichloride)	α -Naphthy- lamine hydrochloride	
(Potassium chloride)	Sodium sulphite, Nickel sulphate, (Ferric sulphate)		(Kerosene)	Naphthalene, (Sodium naphthionate)	
Cryolite	Aluminium sulphate, Chromium amm. sulphate, Nickel amm. sulphate, Aluminium amm. sulphate, Aluminium pot. sulphate	(Amm. phosphate), Sodium silicate, Potassium permang. (Lead nitrate), (Sodium tetraborate)	(Methanol), Acetone, Ethyl acetate, Urea, Motor oil	Pyridine, Naphthionic acid	Sulphite liquor

or three of the weed species. Strict adherence to this basis of classification, however, neglects the magnitude of the partial mortality to the other species. Chemicals in this class were therefore grouped in accordance with the sum of the percentage mortalities to all four weeds. Three groups were defined according to whether the summed mortalities (out of a possible 400) exceeded 350, 300 or 200 respectively (Groups IV, V and VI). The first of these groups was introduced because several of the chemicals failed to kill wild oats completely but gave a relatively high mortality, a result which can probably be attributed to the large discrepancy between the "applied" and "retained" dosages with the large volumes of spray employed. Had a more concentrated solution of these chemicals been applied, a greater proportion of the chemical would have been retained by the leaves and stems, and a complete kill might have been obtained at the same applied dosage. It is doubtful whether chemicals falling in the third class, as defined above, merit further subdivision, since they are not sufficiently toxic to be of any practical value as herbicides. Nevertheless they were divided into two groups (VII and VIII) depending on whether the sum of the percentage mortality to all four weeds was greater or less than 100.

Using these criteria all the chemicals tested were classified into the eight groups shown in Table XII. The first column gives the group number and the definition of the limits of each. In the body of the table the individual chemicals are listed in accordance with the classification used in previous tables. Several individual chemicals are enclosed in brackets; this indicates that the exact position of the chemical is uncertain, either because it was not tested on all weeds, or the maximum dosage was not applied, or for some other reason. It should also be mentioned that the relative toxicity of individual chemicals in one group may not differ significantly for individual members in the group preceding or following it.

Of the 76 chemicals tested seven fall in each of Groups I, III, V, and VII; nine in each of Groups II, IV, and VI; and 21 in Group VIII, which represents chemicals having no useful toxicity. Chemicals falling in the first three groups merit further consideration as herbicides since such substances as sulphuric acid and sodium arsenate, which have been used to a considerable extent in the field, appear in Groups IV and V respectively. Of the acids, chloric and selenic appear to be more toxic than the other mineral acids, indicating that the anion contributes to the toxicity. A fairly large proportion of the arsenicals, chlorates, and cyanides employed had a useful toxicity, but of the 17 halides and sulphates tested, only zinc chloride, zinc sulphate and copper sulphate appear in the first five groups. Again the toxicity of three of the four effective compounds tested in the miscellaneous inorganic group can be attributed to the anion. This may result from the fortuitous selection of chemicals representing the anions and cations generally, but the cost of many salts of other toxic metals would prohibit their use as weed-killers. It appears, therefore, that substances having toxic anions are more likely to be practical herbicides than those based on toxic cations.

It seems reasonable to assume that in most instances a certain amount of the chemical must be absorbed by the plant before it can produce a lethal effect. The amount absorbed must also be conditioned by the physical properties of the substances added, such as its solubility in the aqueous phases of the plant, its volatility, etc. The killing power of a given compound will therefore depend on these properties as well as upon its inherent toxicity after entering the plant. Of the substances listed in Table XII, eight were solids that were too insoluble in water for spraying and had to be applied as dusts. Seven of these were so low in toxicity that they appear in Group VIII. The other member, α -naphthylamine, falls in Group V. From these results, it appears that insoluble, or sparingly soluble solids, will be of little value as herbicides. Seven liquids insoluble in water were applied, and four of these fall in Groups III and IV while the remainder had a lower toxicity. Solubility in water appears therefore to be less important if the substance is a liquid. With reference to volatility, formic acid, benzene and gasoline were fairly toxic, being classified in Groups II and III. The gasoline used, however, was probably not as volatile as the other two substances. Tetralin, aniline and furfural also appear in Group III, but these substances are not highly volatile and must remain on the plant for a considerable period and allow penetration to occur. All of the other liquids, whether applied directly or in solution, were relatively ineffective, and appear in the last three groups.

These results are too meagre to permit a definite statement but it appears that solid substances insoluble in water, and compounds that evaporate readily will be of little value as herbicides applied by dusting or spraying. There are, of course, exceptions to this rule, and in the case of volatile compounds efficacy may be determined by the relative rates of penetration and evaporation. Substances of an acidic nature, such as formic acid, are doubtless absorbed more rapidly than neutral substances and enter the plant before a significant amount of evaporation occurs. With such substances, the observed effect is probably due entirely to the inherent or true toxicity of the compound, rather than to its physical properties.

Residual Toxic Effect in Soil

The residual toxic effect which the chemical may impart to the soil is important, not only because of its subsequent effect on the growth of economic crops, but also from the standpoint of its herbicidal properties for perennials. This class of plant can be eradicated only if the roots are killed, and this condition requires that a sufficient amount of the chemical be transmitted to the roots either through the plant, following absorption by the foliage, or through the soil. Of these two ways in which the roots may be killed, the latter would appear more probable.

In order to determine the residual effect, the crocks were seeded with Marquis wheat immediately after removing the weeds, three to four weeks after treatment. The soil was stirred up, 10 seeds planted, and the moisture content adjusted by weighing. These seeds were allowed to grow for three

TABLE XIII
CHEMICALS HAVING A RESIDUAL TOXIC EFFECT

Chemical	Minimum dosage required to reduce green weight (M.E.D.), lb. per acre	Dosage causing 100% mortality (C.L.D.), lb. per acre	Remarks
Selenic acid	125*	420	*Lowest dosage tested probably higher than M.E.D.
Chloric acid	125*	620†	†Probably higher than C.L.D. as next lower dosage not tested
Sodium chlorate	210	525	
Ammonium chlorate	210*	620	
Barium chlorate	210*	1050†	
Calcium chlorate	210	1050	
	Maximum dosage tested, lb. per acre	Green weight as per cent of checks	
Sodium arsenite	1050	(30)	Gave inconsistent results
Calcium hypochlorite	620	35	
Sodium cyanide	620	60	
Potassium chloride	4200	30	
Copper nitrate	840	80	
Urea	4200	65	
Motor oil	4200	2	
α-Naphthylamine	840	15	
α-Naphthylamine hydrochloride	1050	0	Gave inconsistent results
Creosote	620	(50)	
Sodium benzoate	1050	(10)	

Bracketed values doubtful.

or four weeks and observations were made on the percentage germination. The number of leaves per plant, and the height and green weight of the plants at the end of the growth period, were measured and expressed as a percentage of the growth observed in untreated crocks of the same series. Most of these estimates of growth were correlated with one another, and as the green weight appeared to be the most reliable index, this is the only one reported.

The residual effect was determined only for substances that produced a useful mortality in the spraying tests, namely, those appearing in Groups I to V inclusive (Table XII), with a few exceptions and a few additional members from the other groups. Some of the chemicals produced a toxic condition in the soil over a considerable part of the dosage range, and it was possible to estimate the minimum effective dose (M.E.D.) and the certainly lethal dose (C.L.D.) from the results. Other substances imparted a toxic condition to the soil at only one or more of the higher dosages, so that quantitative estimates of the residual effects were not possible. Of the 35 chemicals tested, 14 showed definite evidence of a residual toxic effect, and three others

sometimes reduced the yield significantly, but gave inconsistent results. Of the remaining substances, 13 showed evidence of increased growth, which suggests stimulation or a nutritive effect, while five did not differ significantly from the controls. Since this investigation was concerned only with the toxic effects, the data obtained with substances which either increased the green weight or had no effect on it are not presented. The results obtained with substances which had a residual toxic effect appear in Table XIII.

This table is divided into two sections, the first includes substances for which sufficient data were available to estimate the M.E.D. and the C.L.D. and the second section includes substances that have a toxic effect, but for which the data were inadequate for quantitative estimates of the toxicity. On the whole, these results are in good agreement with those of Bowser and Newton (3) and Newton and Paul (5). Since the residual effects observed under field conditions will depend to a large extent on the nature of the soil and climatic conditions, any small differences between the results obtained in this study and those reported by the above investigators can probably be explained by variations in these conditions.

It should be noted here that, although the majority of the chemicals tested showed no evidence of residual toxic effects in the soil a few weeks after application, it cannot be said that they did not act through the soil, at least in part, at the time of treatment. In all cases where the rate of reaction of the chemical on the plant exceeds the rate of detoxication of the chemical in the soil, a toxic effect through the soil could be expected. On the other hand, if the toxic effect of a given substance in the soil does not persist for a period in excess of that required for the death of the roots and underground stems of perennials, this substance may be of little value for eradicating this class of weed.

Acknowledgment

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PEACH CANKER INVESTIGATIONS¹

III. FURTHER NOTES ON INCIDENCE, CONTRIBUTING FACTORS, AND RELATED PHENOMENA

By R. S. WILLISON²

Abstract

The relative importance of the different sources of canker varies from year to year. Some, *e.g.*, pruning wounds and injuries from verticilliosis, become less, while others, such as dead twigs and fruit pedicels, become more significant with time. Still others show no definite trends but fluctuate according to conditions.

Leaf scars are vulnerable for a time after leaf fall, because of a temporary absence of wound periderm in the leaf base, but the development of cankers from leaf scar infection is determined by an infrequent coincidence of physiological and meteorological factors.

Some insects such as the oriental fruit moth (*Laspeyresia molesta* Busck.), the shot-hole borer (*Scolytus rugulosus* Ratz.), and the peach borer (*Synanthedon exitiosa* Say.) can cause injuries which frequently become cankered afterwards. The lesser peach borer (*Synanthedon pictipes* G. & R.) is seldom a primary parasite but may stimulate necrotic processes through its destruction of callus in cankers. The peach is most susceptible to canker in the fall, and injuries such as pruning cuts made at that time are much more subject to infection than those made at any other time of the year. The incidence of peach canker and of winter injury can also be increased significantly by prolonging the period of open cultivation. At least three types of winter injury have been observed, all of which may give rise to serious cankers.

From 75 to 85% of the open cankers of all ages overwintering on the tree remain active. There is also a tendency for cankers to become less active with increasing age. Surgical treatment of important cases is of considerable value.

An important phase in the study of the peach canker problem has been the intensive surveys that have been conducted annually in the laboratory orchards from 1929 to 1936 inclusive, a period covering their entire history to date. Although the results of the first four surveys have already been set forth (5), the additional information obtained in the last four years is considered of sufficient interest to warrant a second paper on the same general theme. For present purposes and at the risk of some repetition, it is intended, in the light of more recent observations, to review the status of the various points of origin, and to emphasize the relation of cultural and of pruning practices to the incidence of canker. Other factors, related phenomena and the general behavior of cankers from year to year, are also to be discussed briefly.

Centres of Origin

The percentages of cankers developed from various origins in each year and in the complete series of surveys are given in Fig. 1, for the variety Elberta, and in Fig. 2 for the variety Rochester planted in 1931. It will be noticed that only about 2% of the total number of cankers observed in the Elberta orchard had developed during the first four years. In spite of these small numbers definite trends were noted even then (5). "As the trees

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become older, there is a tendency to shift the responsibility for cankers from pruning cuts to dead twigs, oriental peach moth injuries, scrapes and crotches." This statement still holds in implication, although it will have to undergo some modifications in detail. From the more complete information now available, a few generalizations may be made. During the first two or three years in the history of an orchard, pruning wounds usually outnumber any other form of injury, and thus serve as the main means of entry for canker. Later,

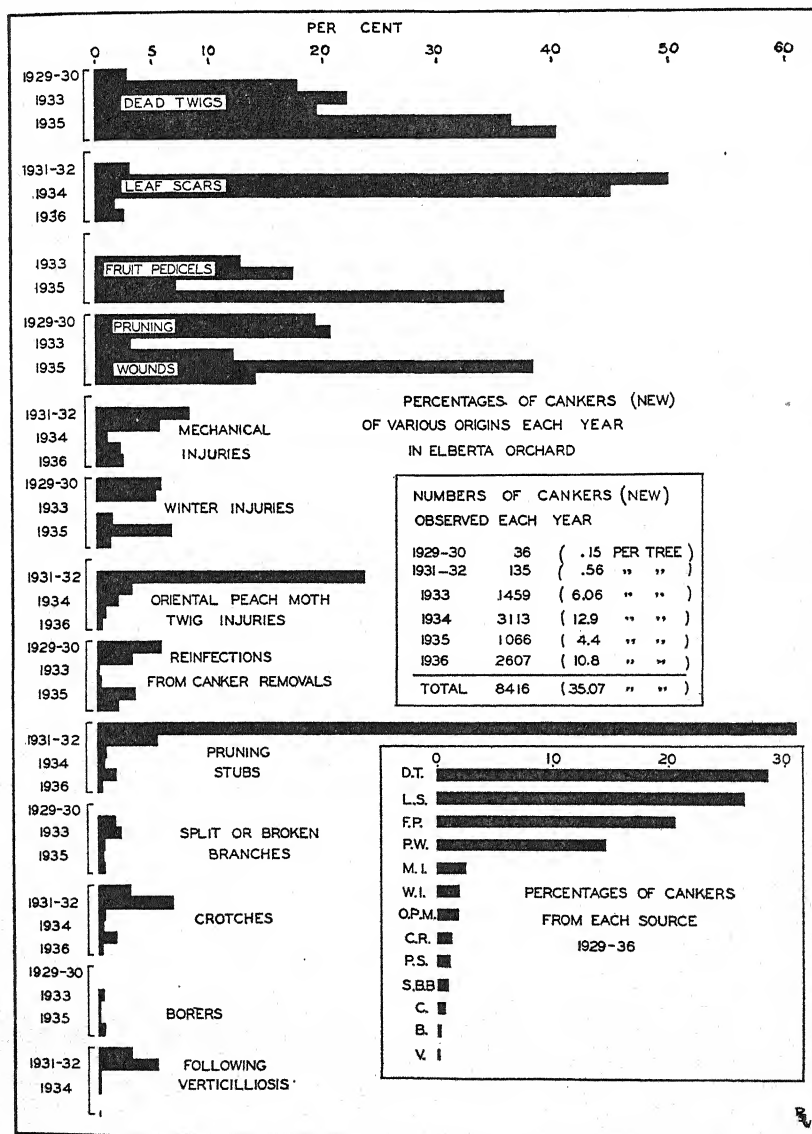


FIG. 1. The relative annual incidence of cankers from various sources in the Elberta orchard. Insets: Upper, the number of new cankers observed each year from 1929 to 1936. Lower, the relative significance of various points of origin in that period.

with the development of a profusely branching aerial system, other points of origin become dominant and either continue to increase in importance from year to year or are superseded in their turn by others. This is evident from the data from both the Elberta and Rochester orchards (Figs. 1, 2)

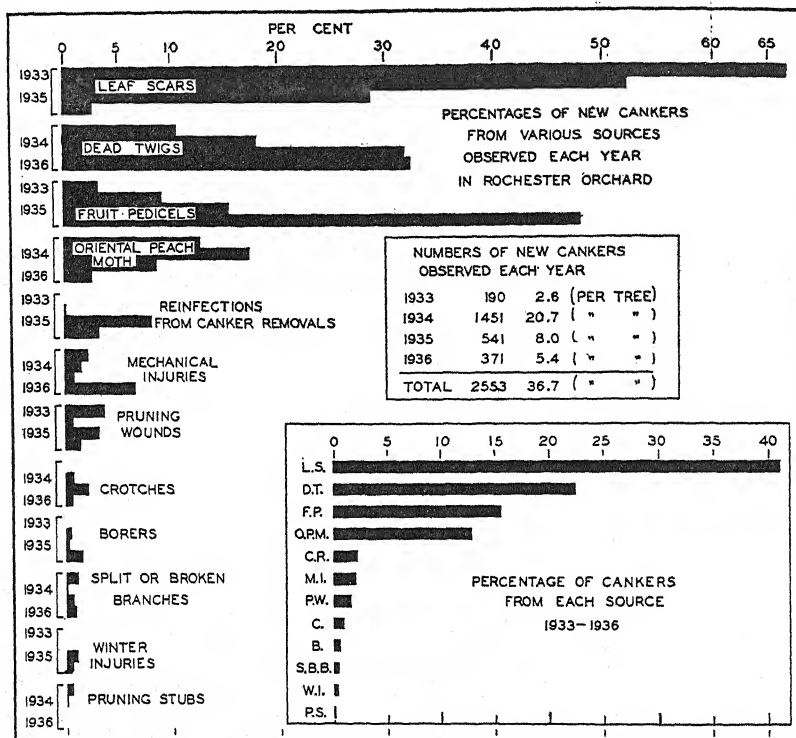


FIG. 2. The relative annual incidence of cankers from various sources in the Rochester orchard. Insets: Upper, the number of new cankers observed each year from 1929 to 1936. Lower, the relative significance of various points of origin in that period.

and particularly in the latter where in 1932, before records were kept, practically all cankers came from pruning cuts. These pruning cuts became of minor significance in later years. The introduction of other factors, which will be discussed at length later, brought about the return of the pruning wound to prominence in the Elberta orchard.

Under the conditions prevailing during the observation period, twig injuries caused by the oriental fruit moth (*Laspeyresia molesta* Busck.) were of greatest relative importance as a source of canker in each orchard during the third and fourth year after planting, though the numerical maxima were reached in 1934 in each case. This suggests that the incidence of cankers of this origin not only may vary with the prevalence of the moth but also declines in importance with the increasing age of the tree, both absolutely and relatively. This is more or less corroborated by a comparison of the occurrence of these cankers in the two varieties (Table I). The evidence here leads to the assumption that the Elberta orchard had passed its period of greatest

susceptibility, and therefore the amount of twig injury in that variety was influenced mainly by fluctuations in the moth populations, the peak of which, on the other hand, occurred when the Rochester orchard was most susceptible.

TABLE I

THE OCCURRENCE OF CANKERS FROM TWIG INJURY BY THE ORIENTAL FRUIT MOTH IN THE VARIETIES ELBERTA AND ROCHESTER FROM 1933 TO 1936

Variety	Numbers of cankers per ten trees			
	1933	1934	1935	1936
Elberta	1.8	2.3	0.6*	0.3
Rochester	3.4	35.4	6.6	1.3
Ratio (E : R)	1 : 2	1 : 17	1 : 11	1 : 4

*Estimated, since removed cankers were counted in Rochester, but not in Elberta in 1935.

So far as these investigations were concerned, verticilliosis and its effects were also chiefly confined to the younger trees and became less important and even non-existent in later years. Observations in other orchards led to a similar conclusion.

On the other hand, dead twigs and fruit pedicels became increasingly significant until they accounted for most of the cankers that occurred after the trees were six or seven years old. Fortunately, these cankers usually appeared on the smaller branches and could be removed without much loss. As indicated in Figs. 1 and 2 the fruit pedicel has appeared as a source of canker in these orchards only during the last four years.

Still other sources, such as mechanical injuries, winter injuries, re-infections following removal of cankers, split or broken branches, crotches, and borer injuries, did not show any definite trends from the point of view either of actual numbers or of relative significance. Annual variations in these appear to be determined by a number of factors independent of the changes brought about by the increasing age or size of the tree.

Brown Rot and Canker

Although no serious outbreaks have occurred in the experimental orchards during the last eight years, it is recognized that, under favorable conditions, *Sclerotinia fructicola*, the brown-rot fungus, is capable of causing great damage to twigs and small branches, following blossom-blight in the spring or brown rot of fruit in the fall. These injuries, particularly the latter, are therefore possible sources of canker which may be of very great importance in some years. However, it should be emphasized again, that, as *S. fructicola* is comparatively short-lived in woody tissues, cankers do not develop at points affected by this fungus unless canker organisms such as *Valsa cincta*, are also present in the lesions (6). Although some of the cankers at fruit pedicels may well have been initiated by *S. fructicola*, in most cases, however, the canker organisms acted as primary parasites, since brown rot was virtually

absent from the orchards in the fall of 1934 and of 1935. Valsoid fruiting bodies were also in evidence in many of these cankers and *Valsa cincta* was isolated in pure culture from a considerable number both with and without fruiting bodies.

Striking evidence of the short duration of the activity of the brown-rot fungus in naturally infected twigs was obtained in 1933. In that year, blossom blight was present, particularly in the variety Mayflower, and the blighted blossoms remained on the tree during most of the summer. In July, about a hundred of these were tagged for future observation and some affected twigs were brought into the laboratory and placed in moist chambers. Four days later, the monilia stage of *S. fructicola* appeared on the dead bark, leaves and blossoms, and on the drops of exuded gum, but underneath the dead bark the callus was quite healthy and in some cases had completely overgrown the original injury. Thus it was possible to isolate the brown-rot fungus from the debris about blossom-blight lesions which had actually healed. In May, 1934, when the tagged lesions on the trees were examined, 80% were either healed or inactive. Three-fifths of the remainder were twigs that had been killed by the blight in 1933 and had died back almost to the parent branch. The few typical cankers that developed from blossom blight were due to secondary infection by canker-producing fungi.

Leaf Scar Infections

The cankers which earlier (5) were designated as originating at dead buds have since been demonstrated to have been leaf-scar infections. One striking evidence of this was the fact that some buds remained alive, and a few actually put out leaves in spite of the cankers around their bases. Upon histological examination, mycelium was found in the leaf traces, in adjacent cortical tissue, in the parenchyma of the leaf gap, and in the cambial region, but the buds either were alive and turgid or had died because of desiccation following invasion of the underlying tissues. There were no indications that infection had occurred through the bud itself, which, after all, is well protected.

Further investigation revealed in the abscission of peach leaves an interesting sequence which helps to explain the vulnerability of the leaf scar. The formation of the abscission layer is not accompanied by the development of cork. Consequently, after leaf-fall, the exposed tissue of the leaf base dies back for a distance of two or three millimetres, at which depth a wound periderm is laid down to form the foundation for a second abscission which occurs early in the following spring. In the process, and before the dead piece drops off, the vascular strands of the leaf base are severed and become occluded by small, bladder-like corky cells developed from the wound periderm. Thus, after the second abscission the leaf scar is fully protected, but there is a period immediately after leaf-fall when the presence of both the dead tissue and the open vessels makes the leaf scar an ideal infection-court. The period of vulnerability varies from year to year and may be short, as in the fall of 1935, when the cork layer was well developed before the advent of winter, or it may last until spring, as in 1934-35, when periderm formation was limited in material examined as late as February.

The presence or absence of periderm, however, is only one of the factors determining leaf-scar infection, since it took place on a large scale only during the fall of 1932 and the succeeding winter. The high percentages of cankers from this source recorded for 1934 in Elberta (Fig. 1), and for 1934 and 1935 in Rochester (Fig. 2) were mainly due to those originating in the outbreak of

TABLE II

SOME METEOROLOGICAL DETAILS CONSIDERED IN CONNECTION WITH THE EPIDEMIOLOGY OF LEAF SCAR INFECTION

Remarks	1931-32	1932-33	1933-34	1934-35	1935-36
Rainfall in August and September (in inches)	6.0	6.0	6.5	6.5	2.9
A. Fall					
1. Dates, first frost and freeze-up	Nov. 6-26	Oct. 13- Nov. 16	Oct. 14- Nov. 4	Oct. 14- Dec. 5	Oct. 5- Dec. 1
2. Number of days	20	34	21	52	57
3. No. of days M.D.T.* 50° F. or higher	13	11	5	15	19
4. No. of days M.D.T. 40-50° F.	6	18	10	19	22
5. No. of days M.D.T. less than 40° F.	1	5	6	18	16
6. Rainfall in inches in 1 and 2	1.59	3.42	1.64	4.3	4.4
B. Winter					
7. Dates†	Nov. 26- Mar. 24	Nov. 16- Mar. 28	Nov. 4- Mar. 28	Dec. 15- Mar. 13	Dec. 1- Mar. 7
8. Number of days	119	132	144	98	97
9. No. of days M.D.T. 32° F. or higher	66	76	47	19	24
10. No. of days max. 40° F. or higher	51	58	37	18	11
C. Periderm (in leaf base)	Well developed††	Comparatively little		Scanty	Well developed

* M.D.T.—Mean temperature for the day.

† Winter is considered to begin when mean daily temperatures start to hover around 32° F., and to end when mean daily temperatures rise more or less steadily above 32° F.

†† By deduction, in consideration of the late first frost, of high temperatures in fall, and of slight amount of leaf scar infection in spite of other favorable conditions.

Weather sequences:

1931-32: Comparatively warm and late fall; followed by a mild winter of about normal length.
1932-33: Early frost; fall, mild but not warm, with temperatures declining fairly gradually, and high rainfall at favorable periods; followed by a long but mild winter.

1933-34: Early frost; fall comparatively cool but changeable with less rainfall than in 1932; followed by a long and very severe winter. The warm periods indicated in 9 and 10 above, were mostly at the beginning and towards the end of winter and were usually not of long duration.

1934-35: Early frost; temperatures very changeable during fall, with considerable rainfall; followed by comparatively short, moderate, but steady winter.

1935-36: Very early frost; first half of fall warm, second half cool, with considerable rainfall; followed by comparatively short, moderate but steady winter.

1932-33 but which had been overlooked in the meantime because of their inconspicuousness. It is, therefore, reasonable to believe that meteorological conditions play an important part in this phase of the problem, and that a combination of factors favoring an outbreak is of comparatively rare occurrence. Some relevant data from the weather records, assembled in Table II, not only indicate a wide range of possibilities but also throw considerable light on the epidemiology of canker formation at leaf scars.

Although it is not possible to state definite limits, there is some evidence that, when mean daily temperatures are above 50° F., particularly for considerable periods, periderm formation proceeds in the leaf bases, and that mean temperatures between 40 and 50° F. are most suitable for infection, which, however, does not readily take place when the mean daily temperature falls much below 45° F. (6). Thus, warm weather after leaf-fall, which is influenced by, but not necessarily coincidental with the first frost, tends to favor the host as in the fall of 1935 and, by inference, the fall of 1931 also. In these cases, subsequent opportunities for infection, which arose in the late fall of 1935 and during the winter of 1931-32, were nullified by the presence of the periderm. On the other hand very variable weather after leaf-fall, as in 1933 and 1934, appears to have permitted little activity on the part of either host or fungus, by reason of the short duration of favorable conditions.

Some infection experiments in the fall of 1934 illustrate the fact that at this time of year apparently slight differences in conditions may be of considerable significance in determining whether infection is to take place, and if so, whether a canker results. On October 5, leaves were removed from a number of twigs either by knife cuts or by tension which broke off the petiole at the abscission layer. Half of the leaf scars thus exposed were inoculated with a spore suspension of *Valsa cincta*. The mean temperature was above 50° F. for 15 of the next 20 days. Natural leaf-fall proceeded from the 14th to the 25th, during which time the mean temperature was above 50° F. for eight days. Periderm was well developed in the artificially induced leaf bases but was scanty following natural abscission. In inoculated leaf bases, the fungus was found in considerable quantity in the dead tissue and had begun to go down the leaf traces only to be stopped by the periderm and masses of wound gum. Incidentally, wound gum was less plentiful in non-inoculated, artificially produced leaf bases and virtually absent in naturally occurring ones. The presence of the fungus had the curious effect of depressing the periderm in the central part of the leaf base, producing a concave surface in place of the almost plane surface characteristic of the periderm in non-infected bases. In the cases cited, although infection actually occurred it was limited in extent, largely by the protective action of the periderm, and no lesions were formed.

In view of the evidence submitted above, the outbreak of cankers following leaf-scar infection in 1932 was in all probability due to the long period in the fall when temperatures, while not high enough to allow much periderm to be laid down, were within the critical zone for infection and were accompanied

by sufficient moisture to permit germination of spores. In addition, the very mild winter provided ample opportunity for the spread of the fungus in tissues in which it had become established, since according to observations in both this and other connections (6) the minimum temperature for growth of the fungus is considerably lower than that for germination and infection.

The Role of Insects

Several insects have been observed in association with canker, either as precursors or as concomitants. The part played by the oriental fruit moth (*Laspeyresia molesta* Busck.) by means of its attacks on succulent twig tips in the spring has already been commented upon (*supra* and (5)). Two other insects, through their ability to attack uninjured bark, have also provided infection courts for cankers. These have been grouped together under the general heading of borers in the lists of origins (Figs. 1, 2). Although the cankers initiated by their activity have been less numerous than in the case of the fruit moth, they may be of greater importance, because of their location on the tree. One of these, the shot-hole borer (*Scolytus rugulosus* Ratz.) produces simple tunnels about a millimetre and a half in diameter in the bark of the trunk or larger branches. These tunnels are without ramifications and penetrate at least as deep as the wood. This insect has generally been considered capable of attacking only weakened trees, but in the last four years of the present investigations it has been found also in quite vigorous, healthy trees, especially those in the neighborhood of dead peach stumps or brush piles. The other insect in this category is the peach borer (*Synanthedon exitiosa* Say.), which, as is well known, works destructively in the crown region just below the soil. This borer was not observed in the experimental orchards until the summer of 1936 when it attacked a considerable number of trees. Some of the injuries showed definite symptoms of being infected with canker.

The lesser peach borer (*Synanthedon pictipes* G. & R.) on the other hand, is found only in the aerial parts of the tree. Its chief significance in the canker problem lies in the fact that its favorite feeding ground seems to be the gum-covered tissues of established cankers, the perennial enlargement of which it aggravates by destroying the callus. As it sometimes also infests non-infected wounds, the lesser borer may also increase the opportunity of infection by keeping the wounds open, although no instances of this were observed.

Pruning Experiments

Although the importance of pruning in its relation to canker has already been discussed at length (5), some more recent observations warrant further examination of this phase of the problem. Since 1932, record has been kept of the number of pruning cuts made each year on each tree in the orchard. With this information it was possible to calculate the percentage of pruning wounds giving rise to cankers and to make a more accurate estimate of the effect of time of pruning upon the incidence of canker (Table III).

TABLE III

THE EFFECT OF THE DATE OF PRUNING UPON THE PERCENTAGE OF CANKERS
DEVELOPED AT PRUNING WOUNDS

Month in which pruning was conducted	Number of pruning cuts	Number of cankers resulting	Percentage of pruning cuts becoming cankered
Elberta orchard			
October-November	16,989	1064	6.26
January	2,576	16	.62
February	2,170	2	.10
March	16,238	24	.15
April-May	51,007	49	.09
June-August	46,935	33	.08
Total (Elberta)	135,915	1188	.88
Rochester orchard			
May, 1934	5,403	17*	.31
May, 1935	7,748	5	.07
Total (Rochester)	13,151	22	.16

* Some of these probably should have been listed as canker removals since it was not always possible to determine in 1935 where cankers had been removed in 1934, in which year canker incidence was high in this orchard (Fig. 2).

Upon the discovery that inoculation in late autumn produced the greatest amount of necrosis (6), it was decided to prune two rows (4 and 7, Fig. 3) in the Elberta orchard in that season. The results (Table III) indicate that wounds made then were also most susceptible to natural infection, since the percentage of cankered fall-pruned cuts was at least ten times as high as that for January pruning and 40 to 78 times as high as that for pruning in any other month of the year. Moreover, the figure given in Table III for the fall pruning is considered to be low. For, while 3.7% of the pruning cuts of October and November 1933 became cankered in 1934, there was an additional 4.4% in 1935. Because of the disastrous results of the first fall pruning, the second was deferred until 1935. From this, there developed in 1935 cankers in 4.3% of the cuts and it is expected that still more will appear in 1937.

Even on the basis of the present figure, there was an average of 22 pruning-wound cankers per tree in the fall-pruned rows. When it is considered, on the one hand, that these were the consequences of only two prunings and that such cankers necessarily occurred on those parts of the tree which it was desirable to retain, and on the other hand, that the cankers resulting from pruning at the proper time of the year were virtually negligible, it is obvious that the salvation or ruination of a peach orchard can, in considerable measure, be determined by pruning practices.

Cultural Practices

The plan of the Elberta orchard, viewed from the east side, in Fig. 3, shows the approximate number of cankers observed on each tree up to and including the survey of 1936, and the arrangement of three plots cultivated until the

middle of June, the middle of July and the middle of August respectively. The squares marked with an asterisk represent replacements, most of which were made in 1931 and 1932. Fig. 4 shows the average number of cankers per tree based, in A, on the number of cankers observed in each plot each

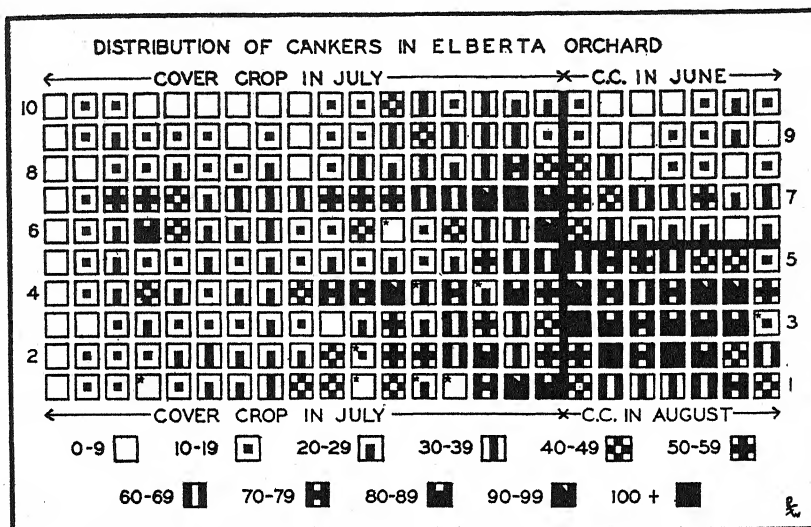


FIG. 3. The numbers of cankers observed on each tree in the Elberta orchard during the period 1929-36, as indicated in the key below the chart. The division of the orchard into three plots receiving different cultural treatments is also shown.

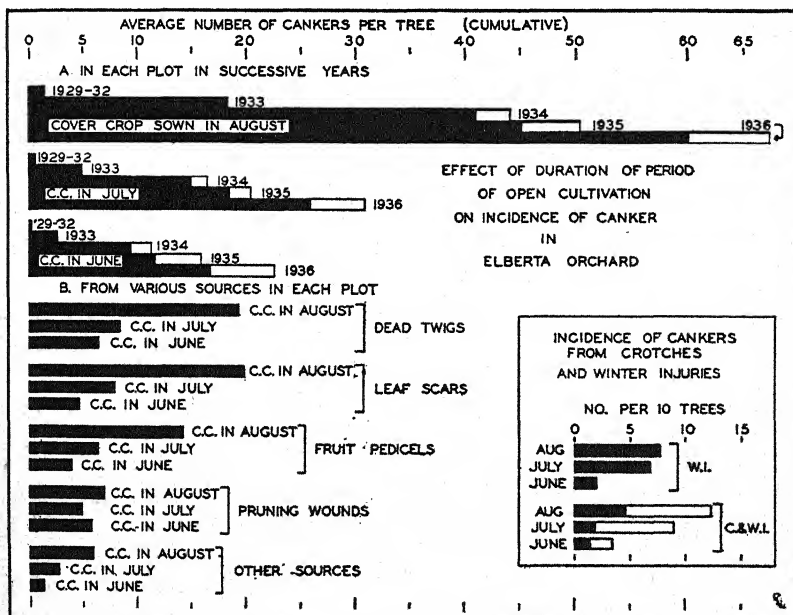


FIG. 4. The effect upon the incidence of canker of cultivating the orchard for different lengths of time during the growing season. Variety Elberta.

year added to the total of those originating in previous years, and in B, on the total number of cankers of various sources in each plot from 1929 to 1936 inclusive. In A, the blank spaces in the columns for the years 1934, 1935 and 1936 represent cankers from pruning wounds, the incidence of which is not affected by cultural practice (see also B, Fig. 4).

It is strikingly evident that the longer the orchard is cultivated during the summer, the greater is the amount of canker. This effect, which has already been mentioned (5), has been consistent during the last four years, not only *in toto* but also in cankers from each of the various points of origin except the pruning wound. It will also be observed (Fig. 3) that the densest concentration of cankers occurred in the central part of the August plot, while the opposite was true of the June plot. That is to say, the outer rows of each plot appeared to be affected to some extent by the conditions in the adjacent one. A roadway along the east side of the orchard also tended to reduce the amount of canker in Row 1 of the August plot though it apparently had little effect on the July plot.

On the basis of the data given in Fig. 4, the proportion of incidence was 1 : 1.4 : 3 in the June, July and August plots respectively. It would appear then, that cultivation until the middle of August had greater influence in increasing the incidence of canker than cultivation until July. There was, however, a lack of uniformity in the distribution of cankers in the July plot (Fig. 3) attributable in part to differences in growth rates brought about by the more pronounced differences in topography and soil fertility in that plot. It is probable, therefore, that the above estimate for the July plot may be slightly lower than would be applicable to general practice. But even if only that section of the July plot where cankers were most prevalent is taken into account, that is, a block seven trees wide extending across the orchard next to the other two plots, the proportion becomes only 1 : 2.2 : 3. At that estimate, the critical time for stopping cultivation still remains between June 15 and July 15. In any case, there is ample evidence that the susceptibility of the peach tree to canker can be profoundly modified by a factor as readily controllable as the date of sowing a cover crop.

In a consideration of the effects of cultural practices, crotch cankers and trunk cankers following winter injury are of particular interest (see inset, Fig. 4). These cankers, because of their position, are of much greater importance than their numerical status (Figs. 1, 2) would indicate, since in most cases they cannot be removed and can affect the whole tree or a large part of it. In the plot cultivated until June 15, only one tree in three was subject to one or other of these cankers, in comparison with 87% of the trees in the July plot, while, in the August plot, assuming uniform distribution, three-quarters of the trees had one or other and the remaining quarter both. Moreover, the proportion of cankers from winter injury in the various plots implies a similar proportion of winter injury, whether infected or not. This, of course, suggests a possible means of avoiding or reducing such injury and adds further weight to the argument for early cessation of cultivation.

Winter Injury

In the course of the present investigations, at least three types of winter injury have been encountered. The first, occurring in the winter of 1930-31, was a crown injury or winter "sunsald" induced in the early spring by cold weather following a warm period during which the crown became active. Several trees were girdled and died in May or June, in addition to those with lesser injuries, most of which appear as cankers in Fig. 1.

A more serious outbreak of winter injury in the winter of 1933-34 took the form of frost cracks in the bark apparently caused by tensile strains developed as a result of unequal contraction during periods of very low atmospheric temperature in February. These gave rise to the cankers recorded for the years 1934 to 1936 (Figs. 1, 2). While no trees in the experimental orchards were killed, a number of large branches either succumbed or were badly affected because the resulting cankers on the trunk either cut off or seriously impaired their supply of water and nutrients. During the same winter, orchards in other parts of the Niagara Peninsula, where the temperatures were lower than at St. Catharines, suffered severe losses from direct low temperature injury to buds, twigs and even large branches.

The third form of winter injury appeared after the winter of 1935-36, during which the soil temperatures reached the lower limits of tolerance for subterranean parts. Several trees suffered girdling but others were affected on one or two sides only. It should be mentioned in passing that damage of this type was most severe in those portions of the experimental orchard where soil fertility was low, and that a similar correlation existed in other orchards to which attention was called.

Although somewhat aside from the main purpose of this paper, a brief discussion of the symptoms exhibited by girdled trees and branches seems to be appropriate here. If girdling, whether by winter injury or by canker, takes place before bud break, the tree or branch usually, though not always, puts out its leaves which, however, rarely attain full size and are usually more or less chlorotic. The affected foliage may also show symptoms of mineral deficiency of one kind or another not observable in the rest of the orchard or tree. Depending upon the extent of the interruption of water supply, the leaves may remain alive throughout the growing season or they may flag at any time during hot weather and drop off shortly afterwards. Cases of winter injury have been observed, especially in 1936, where the phloem tissues of the trunk and large branches turned brown from the base upwards, so that it was possible for the upper branches to have been dead or dying and yet to have had undischored bark. Later, of course, discoloration followed, unless desiccation of the tissues was rapid. The symptoms appearing on trees attacked by *Verticillium* are somewhat similar to those following girdling.

The resemblance of the above-mentioned symptoms to those described by European workers (1, 2, 3, 4) for "apoplexy" of stone fruit trees, leads to the suggestion that that trouble also occurs in Ontario. If so, it is probably due to a number of entirely different causes, acting either independently or

in various combinations. That opinion is also current among aforementioned European authorities, but they have not suggested that canker may sometimes be implicated.

Sunscauld

Since the completion of the 1936 survey, there has appeared an additional source of canker, which, in some orchards and in exceptional seasons, may be of considerable significance. During the latter part of August, 1936, depressed areas were observed in a few trees, in the bark on the upper side of larger branches extending to the northeast. The bark in these areas was dead and brown, in some cases as deep as the cambium but in others only part way. The outer layers of xylem were also browned. In other trees, the injury was limited to brown flecking, speckling and streaking of the bark tissues. The same minor injury was also present just outside the borders of the depressed areas in the more severe cases. Dead areas of the same type were also found on the west side of the trunks of two trees. By April, 1937, some of the branches on which the bark was killed had become infected with a species of *Valsa* and the necrotic areas had undergone considerable increase in size. Non-infected lesions, on the other hand, appeared to have remained unchanged.

Upon histological examination, it was found that, in bark only partially affected, the cortex, phloem, phloem parenchyma, and fibres were more or less collapsed and filled with a yellowish mass of wound gum, while the medullary rays were for the most part uninjured. Where the bark, or part of it, was dead, a well developed wound periderm separated the dead from the living tissues, which were usually partly affected also. A lesser amount of wound periderm had also been formed around some of the worse areas in the latter.

This type of injury was confined to that section of the orchard where the trees were in a poor general state of health due, in part at least, to low soil fertility. The nature of the damage and its position on the tree suggest that it was caused by a sunscauld induced during the extreme heat of July, 1936, in trees already weakened from other causes. A similar condition was also observed on some old plum trees on the laboratory farm.

The Perennial Activity of Cankers

As most peach cankers are more or less perennial, an account of field observations such as this would not be complete without some remarks on their behavior from year to year. During the present investigations, case histories of all cankers were followed until they either were removed from the tree or were healed by the complete overgrowth of callus. The data pertaining to this phase of the problem are summarized in Fig. 5. On the left-hand side are given the data for cankers recorded as inactive or healed from year to year and calculated as a percentage of the number of cankers originating each year (inset in each group). The graphs, of which the base line is at the right-hand side, represent the percentage of active cankers in those which were on the tree, but not healed, during the preceding winter. Both categories are

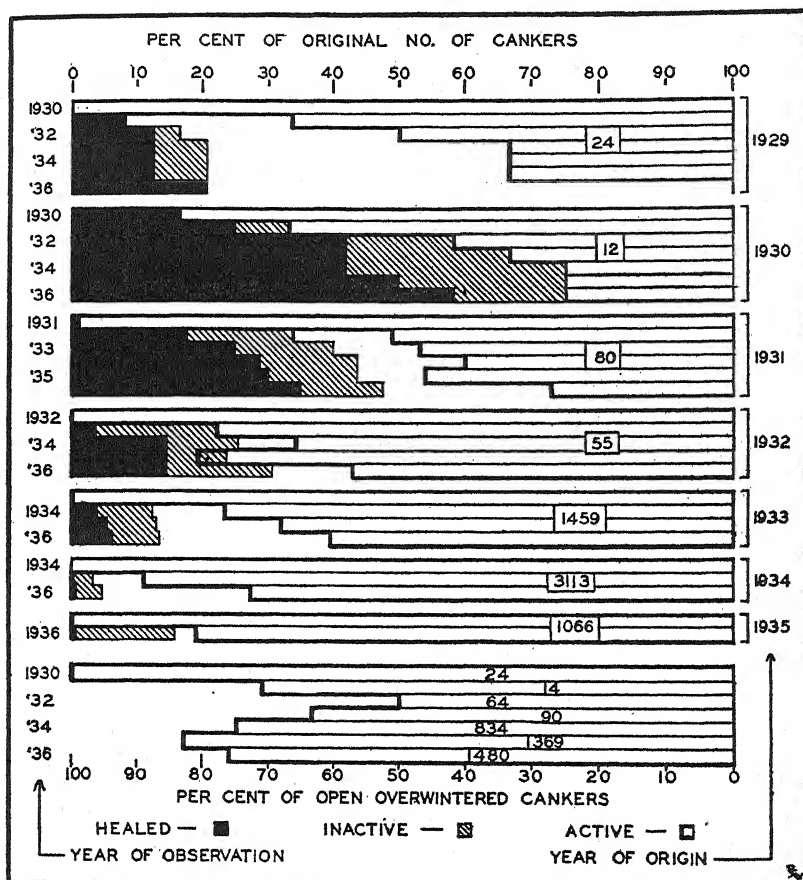


FIG. 5. At the left, the percentage of the cankers of each year of origin becoming inactive and healed in successive years. At the right, the percentage of active cankers in those remaining open on the tree during the winter preceding the year of observation, arranged according to both year of origin and total numbers overwintering. Variety Elberta.

classified according to their year of origin in the first seven groups, and in the lowermost group the active cankers are represented on a cumulative basis in terms of the number of open cankers of all ages remaining on the tree each winter. These numbers are given in the columns for the respective years. The spaces between the two sets of graphs are due to the fact that some cankers were removed each year, either by excision or by being healed. Since 92% of these were active at removal, the estimates as calculated for the inactive cankers probably approximate more closely what would be expected to happen if no cankers were removed. On the other hand, the graphs for the active cankers give a more accurate account of what actually occurred in overwintering cankers.

It is evident in each age group that a certain number of cankers which were active in one year became inactive during the succeeding one and thereafter proceeded to heal over. There were occasions, however, when cankers

resumed activity, some of them even after being healed. The effect of this is apparent (Fig. 5) in 1934 in the 1931 series and in 1935 in the 1932 series, while in other instances, it was balanced by the opposite behavior of other cankers. In still other cases where cankers became inactive the original lesion remained so, but a new one appeared nearby, suggesting the new emergence of an old canker. The tendency to inactivity with increasing age was expressed not only numerically, but also spatially, for the greatest amount of necrosis was usually produced by a canker during its first year or two. But since there were exceptions, this rule, while generally applicable, is scarcely a safe guide for prediction in particular cases. In addition to these considerations, there is some evidence that trees show more recuperative ability when they are young and vigorously growing than they do later. This is in accordance with the observation that the rate of closure of wounds depended upon the growth rate of the branches on which they were found (6). Whether a canker will become inactive is also determined by the same factor as well as by the foothold gained by the causal organism. Besides, the low percentage of inactive cankers in the later age groups may be also partly due to the occurrence of the majority of cankers on the smaller branches.

With regard to the data summarized in the last section of Fig. 5, it will be observed that in later years when there were large numbers of cankers overwintering the percentage of active ones fluctuated over a narrow range (75 to 83%). This provides a basis for estimating the minimum to be expected to become perennial. The wider range of values obtained during the first four years may be due in part to the small number of cankers under consideration and in part to the operation of the age factor mentioned above.

Closely connected with this aspect of the problem is the effect of surgical treatments upon the subsequent behavior of cankers. During June and July of 1934 and 1935, 187 cankers on trunks and large branches were thoroughly cleaned. Various water-proofing preparations, mostly asphaltic in nature, were applied to 85 of these. Approximately half of the treated cankers were previously disinfected with a 1 : 500 solution of mercuric chloride. In the two years' experiments, only 37.7% of the treated cankers showed further activity. Since the use of a single preparation rendered the treatments more strictly comparable in 1935, the results of that year's experiments are of particular interest. The percentages of cankers active in 1936 were 33.3 for disinfected, treated cankers, 58 for those treated with asphalt alone, and 66.6 for those cleaned but otherwise untreated. The waterproofing apparently acts in a double capacity, first to promote callus growth by curtailing water losses, and second, to prevent leaching of the disinfectant by rains. It is also probable that, in cankers that remain active despite treatment, the causal fungus was deep-seated and thus beyond the point to which the disinfectant could penetrate effectively. It may be concluded then, that surgical methods combined with wound dressings, particularly when disinfectants are also employed, are of most value in the borderline cases and in hindering re-infection.

Acknowledgments

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A STUDY OF *BACTERIUM GLOBIFORME* CONN IN SOILS DIFFERING IN FERTILITY¹

BY C. B. TAYLOR² AND A. G. LOCHHEAD³

Abstract

Observations were made of the abundance of *Bacterium globiforme* Conn in three soils that had been subjected to different fertilizer treatments. It was found that the organism was as numerous in a soil of low fertility which had been cropped continuously for 25 years without application of fertilizer as in plots of greater crop-producing power receiving farmyard manure and artificial fertilizer. Freezing of the soil under field and artificial conditions had no significant effect on the numbers of the organism.

From the soils, 110 cultures of *Bact. globiforme* were isolated; ten strains were studied in detail. All showed characteristic metamorphosis from rod to coccus though variations in cell size and time-rates of change were observed. The change of shape is not merely a shortening of the rod until the organism becomes spherical, but involves a swelling of the rod followed by a fragmentation leaving ovoid bodies which become cocci.

Introduction

For many years it has been the intent and hope of soil biologists to correlate or measure certain soil constituents and fertility by some microbiological method. Some success has been achieved in such directions as the estimation of available phosphorus by the *Azotobacter* soil plate method of Winogradsky and Ziemiecka (10, 11) and the *Cunninghamella* method of Mehlich, Fred and Truog (8). A method for indicating soil deficiencies, particularly of potash, depending on the growth of *Asperigillus niger*, has been extensively used by Niklas and associates (9).

The narrower and more fundamental question of relating any one type of organism to soil fertility in general was left open until Conn (3) isolated from fertile soils a type of bacterium which he found to be absent from two less productive soils. The organism, *Bacterium globiforme* (classified by Bergey (1) as *Achromobacter globiformis*), has a very interesting morphology. In a 24-hr. culture on an agar slant it appears as short rods about 0.6 to 0.8 μ long, but as the culture ages the cells become spherical until the culture has the appearance of a micrococcus. Not only was this type of organism missing from the two soils, a Volusia silt loam and a Hoosick coarse sandy loam, but no growth could be obtained when the organism was inoculated into sterilized samples of the soils. The soils were decidedly acid, but the addition of suitable carbon and nitrogen sources as well as lime was found to be necessary to produce growth of *Bact. globiforme*. Conn suggests that the inability of these soils to support growth of *Bact. globiforme* is associated with their relatively low productivity. It is not implied that this organism is necessary for the good growth of plants but that generally crops cannot thrive in a soil deficient in the substances required for the nutrition of *Bact. globiforme*.

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Further work was carried out by Conn and Darrow (4) on the same two soils. Samples of the soils were sterilized and treated with many different carbon and nitrogen sources, separately and in combination. A suspension of *Bact. globiforme* was added, the soils incubated and the prevalence of the organism estimated roughly by direct microscopic examination of stained soil smears. Growth of the organism was obtained by the addition of ammonium salts, nitrates and certain forms of organic nitrogen. The authors concluded that although the total nitrogen of the soils was high, available nitrogen was lacking. As growth of *Bact. globiforme* was also obtained when simple non-nitrogenous compounds such as hydroxides, sulphates and phosphates were added to the soil, it was supposed that such compounds rendered part of the unavailable nitrogen available for the use of the bacteria.

In later work Conn and Darrow (5) measured the efficiency of *Bact. globiforme* in breaking down glucose and sucrose and also studied its nitrogen consumption. It was found to be a very economical user of sugar and that 60–90% of the nitrogen supplied was utilized as cell substance. No direct loss of nitrogen was evident. The authors conclude that *Bact. globiforme* retains in the soil nitrogen which has been converted into soluble forms by other organisms and which otherwise would have been removed by drainage or utilized by plants. The morphology of the coccus-forming rods and their relationship to other soil organisms is discussed. These coccus-forming rods of which *Bact. globiforme* is the prototype, previously included in the "punctiform-colony-forming group", are considered to represent a large proportion of Winogradsky's "autochthonous" (indigenous) group.

The work on the subject is summarized briefly by the following points:

- (1) That there exists in fertile soils a type of organism having the unusual physiological property of changing from a rod form into a coccus form.
- (2) That although the organism is not necessary for the good growth of plants it is more abundant in good soils than in less productive soils.
- (3) That the relation of the organism to soil fertility is based mainly on the available nitrogen fraction present.

These findings are extremely important. If there does actually exist some easily isolated organism whose presence or absence in soil indicates, even to some degree, the crop producing power of the soil, or will aid in differentiating types of soil, the soils scientist will have at hand a new method for soil survey.

Experimental

The observations here reported were made in the course of a study of the incidence of various types of bacteria in three soils that had been subjected to different fertilizer treatments. A previous study (6) of the same areas had shown differences between soils of low and high fertility in their ability to support certain strains of *Rhizobium* and *Azotobacter*. The chief finding was that the relative numbers of *Rhizobium meliloti* were consistent with the crop yields of all three soils throughout a four-year crop rotation. Later

investigations, dealing with a qualitative study of the microflora as a whole, showed the presence of *Bact. globiforme* in all soils and special attention was given to the incidence of this organism in the areas studied.

The soils used in the work were taken from plots in a sandy-loam area supporting a rotation of clover, oats, mangels and timothy. The experiments were started following the crop of timothy and in one case following mangels. The plots had received the following manurial treatments for the previous 25 years.

Soil N—No fertilizer.

Soil X—15 tons farmyard manure, applied to mangels.

Soil Y—100 lb. nitrate of soda, 300 lb. superphosphate, 75 lb. muriate of potash to mangels; 100 lb. nitrate of soda to oats, clover and timothy.

The soils contained approximately 0.11%, 0.16% and 0.13% of nitrogen respectively and had reactions slightly more alkaline than pH 7.0.

Composite samples were taken from the 2-4 in. layer on two occasions in the autumn before the soil was frozen, and once in February when the soil had been permanently frozen for approximately three months. Part of the second sample taken in the autumn was frozen in a refrigerator at 0° F. for two months and then re-examined.

The soil was well mixed, sifted and plated at once. In the first experiment plain gelatin medium used by Conn was employed; but as it gave such low total counts it was replaced by soil extract agar prepared according to Löhnis (7). Plates were incubated for 12 days at 28° C. (gelatin for 6 days at 18° C.). To provide quantitative data all colonies on representative sectors of plates from each sample were picked off for morphological and cultural study. After 24 hours' incubation subcultures were examined and gentian violet preparations made from any showing growth. After a further four days' incubation the cultures were re-examined, the morphology of the organisms at the two different times compared and all cocci-forming rods separated for more detailed studies.

The ability of the organisms to utilize dextrose, reduce nitrates to nitrites, and liquefy gelatin was determined. As in Conn's findings it was noted that more often than not the buffer action of the medium masked the appearance of acid production in dextrose broth. In order to determine the production of small amounts of acid without the use of a purely synthetic medium, a weakly buffered semi-solid soil-extract medium containing 1% glucose was substituted. A semi-solid soil-extract nitrate medium proved to be satisfactory for the determination of the production of nitrite.

Incidence of *Bact. globiforme* in Three Soils

From the four samplings 110 cultures of cocci-forming rods were isolated, all of which were identified as types of *Bact. globiforme* Conn. The approximate calculated numbers per gram of oven-dry soil are shown in Table I,

TABLE I
INCIDENCE OF *Bact. globiforme* IN SOILS OF DIFFERENT FERTILIZER TREATMENT

Soil		Crop yield (tons per acre)		<i>Bact. globiforme</i> —millions per gram, and percentage of plate count									
Treatment	Previous crop	1936	Av. 25 years	Sept. 25, 1936 (tap water gelatin)		Nov. 12, 1936 (soil extr. agar)		Jan. 12, 1937* (soil extr. agar)		Feb. 23, 1937† (soil extr. agar)			
				No.	%	No.	%	No.	%	No.	%		
N, no fertilizer X, manure Y, artificial fertilizer	Timothy	1.65	2.01	5.8	35.8	10.5	11.6	7.7	6.7	6.9	7.7		
	Timothy	2.85	3.10	2.3	25.7	7.5	7.8	3.9	3.3	10.2	9.2		
	Timothy	2.51	2.66	4.4	32.5	9.1	7.7	9.2	7.7	8.1	6.1		
N, no fertilizer X, manure Y, artificial fertilizer	Mangels	2.59	7.99	—	—	18.0	14.6	—	—	—	—		
	Mangels	29.03	22.72	—	—	14.4	10.3	—	—	—	—		
	Mangels	25.12	20.91	—	—	14.7	11.6	—	—	—	—		

*Soil from Nov. 12, 1936, held at 0° F. in refrigerator.

†Soil direct from field, frozen.

together with the crop yields of the corresponding plots for the previous year, and average yields for 25 years. From the data there is no indication of any relation between the incidence of *Bact. globiforme* and the productivity of the soils in question. It is found that greater numbers occur after mangels than after timothy, which suggests a possible influence of the crop on the abundance of the organism. *Bact. globiforme* comprised a larger percentage of colonies from the tap water gelatin plates used in the first sampling (Sept. 25, 1936), indicating that this medium is more selective for this organism than soil extract agar.

Among the 110 cultures, several different strains were found which were differentiated from one another by their action on dextrose, reduction or non-reduction of nitrates, and in some cases by the production of pigment. Some of the strains were isolated on one or two occasions only. Conn (3) noted several different strains, some of which also appeared only once or twice. His most common strain is described by Bergey (1). The description is condensed as follows:—

Achromobacter globiformis (Conn) Bergey *et al.* (*Bacterium globiformis* Conn): Short rods, 0.4 to 0.6 by 0.6 to 0.8 μ , becoming coccoid in older cultures.

Gelatin stab: Slow crateriform liquefaction.

Agar slant: Filiform, flat, smooth, soft, translucent, glistening, with characteristic sheen.

Nitrates reduced to nitrites.

Dextrose, sucrose, mannitol and, less readily, lactose are used as sources of carbon and energy when grown in synthetic media.

On comparing the 110 cultures isolated, it was noted that Conn's most prevalent type, as described above, was certainly not the most common. All of the 110 cultures showed crateriform gelatin liquefaction, although there was a considerable difference in the time taken to liquefy. Sixty-nine cultures produced acid from dextrose, the rest showing no change or a slight alkalinity. Only 30 reduced nitrates in any degree whatsoever. Table II shows the distribution of cultures according to their action on dextrose and nitrate.

TABLE II
ACTION ON NITRATES AND DEXTROSE OF 110 STRAINS OF *Bact. globiforme*

Nitrates reduced		Nitrates not reduced	
Acid from dextrose	No acid from dextrose	Acid from dextrose	No acid from dextrose
17	13	52	28

Comparison of Different Strains

More detailed study was made of ten strains which showed different reactions to dextrose and nitrate or were chromogenic. Tests were made as to their ability to ferment different carbohydrates added in 1% concentration to semi-solid soil extract medium. The results, shown in Table III, indicate considerable variation in the reactions of the various strains.

TABLE III
REACTIONS OF TEN STRAINS OF *Bact. globiforme*

Culture	Chromo- genesis	Nitrate reduction	Acid produced from					
			Dex- trose	Lac- tose	Mal- tose	Suc- crose	Man- nite	Salicin
NM4	—	+	+	+	—	—	—	—
XM51	Yellow	+	—	—	—	—	—	—
YT34	—	+	+	—	—	+	+	+
YF2	—	+	+	+	—	—	—	—
YG12	—	—	+	—	—	+	—	—
NG53	—	—	+	—	—	+	+	+
XG11	—	—	+	—	—	—	+	—
YT54	—	—	+	—	—	+	+	—
YG11	Yellow	—	+	+	—	+	+	—
29	—	—	+	—	—	+	+	—

Morphological comparisons were also made on nutrient agar slants incubated at 28° C. At four different periods—17 hr., 41 hr., 65 hr., and 6 days—gentian violet smears were prepared from the cultures and examined. Initial and final cell measurements were made and the percentage of cocci present at the four periods was noted.

From Table IV it can be seen that there was very little difference in cell length at the beginning of the experiment but that there was considerable variation in the size of the cocci on the sixth day, not only between different

TABLE IV
PERCENTAGE COCCI AND CELL SIZE DURING GROWTH OF TEN STRAINS OF *Bact. globiforme*

Culture No.	Cocci present in culture					
	17 hr.		41 hr. %	65 hr. %	6 days	
	%	Mean cell length, (μ)			%	Cell diam., (μ)
NM4	0	1.6	10	50	95	0.7-1.0
XM51	80	1.8	100	100	100	1.0
YT34	10	1.8	40	60	95	0.9-2.0
YF2	20	1.6	90	90	95	1.0
YG12	10	1.8	20	85	95	0.7-1.2
NG53	2	1.4	95	100	100	0.8-1.0
XG11	5	1.4	60	80	80	0.8-1.5
YT54	0	1.4	100	98	100	1.0
YG11	5	1.9	80	90	100	0.7
29	0	1.6	20	40	90	0.9-1.5

strains but between individuals in the same culture. It is also apparent that the rate of change from rods to cocci varies considerably with the strain. In Strain XM51, 80% of the cells present were cocci after only 17 hours' incubation, whereas Strains 29 and NM4 had only 40% and 50% of cocci present respectively, after 65 hours' incubation.

The metamorphosis of the rod to the coccus is most interesting and is a striking example of pleomorphism (Plate I, Figs. 1-6). Conn (2, 3) described the change as merely a shortening of the rod until the cells become spherical, but from periodic examination of the cultures during their metamorphosis the process would appear to be less simple. The rod generally becomes granular and begins to distend, usually at one extremity. The swelling continues until the rods are club-shaped and bent, after which the remainder of the rod breaks away leaving an almost spherical body with a faint tail (Plate I, Figs. 2 and 5). Later the tail disappears and the cell has the appearance of a perfect coccus.

Discussion

Conn's discovery of the existence in the soil of a group of organisms, which are rod-shaped in young cultures and later change into cocci has been confirmed by the isolation of more than 100 cultures of this group. These can be divided into several different strains of *Bact. globiforme* Conn.

As far as the soils in question are concerned no relation has been noted between the abundance of this organism and the productivity of the soils which were specially chosen by reason of their difference in crop-producing power. It is true that the poor soil (N) used in the experiment differed from the Volusia and Hoosick acid soils with which Conn worked, and hence it is more than possible that the absence of *Bact. globiforme* may be confined to unproductive soils of definite types. Conn (3) himself has observed that in a soil of low productivity (Dunkirk fine sand) good growth of this organism could be supported.

Conn and Darrow (4) suggest that fundamentally the growth of the organism is dependent on the available nitrogen present, and that poor soils, lacking available nitrogen, fail to support growth. Their experimental evidence, such as the facility of the organism to multiply when there are added to the soil easily-available nitrogen sources, or certain hydroxides, sulphates, carbonates and phosphates which are claimed to make the soil nitrogen available, would tend to support this view. However if this is the case, mere steam sterilization alone might be expected to release sufficient available nitrogen for the needs of the organism, though in point of fact no growth was obtained by such treatment. Conn and Darrow also suggest that the nitrogen may be unavailable because of being adsorbed by the soil colloids, but as steam sterilization of soil has such a drastic action on the colloids it may be doubted whether this supposed phenomenon is related to the growth of *Bact. globiforme*. Types of soil, such as those used by Conn, which have a high nitrogen content and whose chemical analysis suggests high productivity, but which, in view of crop-yielding power, are actually poor soils, are common in



1. *Bact. globiforme* (Str. NM4), 12-hour culture on nutrient agar, Gram stain, showing rod stage. $\times 1150$. 2. *Bact. globiforme* (Str. NM4), 24-hour culture in soil extract, Gram stain. Intermediate stage, showing formation of coccoid bodies from rods. $\times 1150$. 3. *Bact. globiforme* (Str. NM4), 6-week culture on nutrient agar, Gram stain, showing cocci. $\times 1150$. 4. *Bact. globiforme* (Str. NG53), 12-hour culture on nutrient agar, Gram stain, showing rod stage. $\times 1150$. 5. *Bact. globiforme* (Str. NG53), 24-hour culture in soil extract, gentian violet stain. Intermediate stage, showing formation of coccoid bodies. $\times 1150$. 6. *Bact. globiforme* (Str. NG53), 60-hour culture on nutrient agar, Gram stain, showing cocci. $\times 1150$.

certain areas of eastern Canada. It is therefore hoped to pursue the investigation further and to study more closely the factor or factors which affect the occurrence and growth of *Bact. globiforme* in soils of low productivity.

It is not intended at this time to discuss the relationship of *Bact. globiforme* to the so-called autochthonous group of organisms in the soil but it is worthy of notice that this type of organism represents some 10% of the organisms which are capable of being isolated by plate-counting methods and less than 1% of the total cell count obtained by microscopic examination. *Bact. globiforme* and its variants are an easily isolated group of soil organisms, owing to the rapidity of growth on nutrient agar and to the fact that they represent the largest group of gelatin-liquefying organisms present on plates from soil. That *Bact. globiforme* is resistant to cold is indicated by the fact that no appreciable decrease in numbers was obtained in a sample held at 0° F. in a refrigerator for two months or from samples which had been frozen continuously in the field for approximately three months.

The morphology of the organism is even more interesting than previously described and its elementary life cycle makes it worthy of more intense study.

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FLAX STUDIES

III. A REFRACTOMETRIC METHOD FOR THE ESTIMATION OF IODINE VALUE OF RAW LINSEED OIL¹

BY F. H. LEHBERG² AND W. F. GEDDES³

Abstract

A rapid refractometric method for the estimation of the iodine value of freshly prepared linseed oil is described. This involves expression of the oil at laboratory temperature from finely ground flaxseed, determining the refractive index at 25° C. and converting to Wijs iodine value by means of the regression equation expressing the relation between these variables. In a study of cold pressed linseed oils prepared from 339 samples of sound Canadian flaxseed varying in Wijs iodine value from 153 to 202 units, a correlation of 0.980 between refractive index and Wijs iodine value was found which permits the estimation of iodine value with a standard error of prediction of 2.1 units.

Ethyl ether extracts obtained by the usual extraction procedure for determining oil content are not recommended, owing to differences in the characteristics of the oil and a lower degree of association between refractive index and iodine value.

Since the refractive index of linseed oil and its relation to iodine value are influenced by free fatty acids, oxidation and polymerization, the method is not applicable to commercially prepared oils nor to flaxseed which has heated or become musty.

The method is considered sufficiently accurate for surveys of the quality of flaxseed produced in different districts and of new varieties and hybrids submitted by plant breeders. It should also prove of considerable utility to linseed crushers for securing a measure of the intrinsic drying value of their raw material.

Introduction

The commercial value of flaxseed depends primarily on the quantity and quality (drying properties) of the linseed oil it is capable of yielding. The drying properties depend upon the extent and rate of oxygen absorption, both of which are associated mainly with the total degree of unsaturation. While the rate of oxidation of linolenic, linoleic and oleic acids decreases in the order named, and hence is influenced in part by the relative proportions of these acids present as glycerides, the total degree of unsaturation, as measured by iodine value, has been found to give a valuable and fairly reliable index of the drying quality of linseed oils, and is used extensively in commercial practice.

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The quantity and quality of the oil in flaxseed vary over wide ranges, depending upon variety and the climatic conditions under which it is produced; for example, surveys conducted by this laboratory on commercial flaxseed marketed in Western Canada during 1934, 1935 and 1936, reveal a range in oil content from 35.2% to 45.2%, on a dry matter basis, and in iodine value from 168.5 to 201.7, the lowest values being obtained in 1936 owing to the unusually dry weather and high temperatures which prevailed during the growing season. These variations represent wide differences in commercial value and it has been shown by Coleman and Fellows (6), Birchard (5), Geddes (8, 9) and Geddes and Lehberg (11), that the physical factors used officially for the grading of flaxseed in the United States and Canada fail to differentiate flaxseed satisfactorily on a quality basis. However, the conventional chemical methods for determining oil content and quality are not suitable for routine grading purposes, and convenient rapid and accurate methods are required to place the grading of flaxseed on a scientific basis. Such methods would also be of great utility in the development of new varieties, in zoning the producing areas, and in the linseed oil industry.

These considerations have stimulated research leading to the development of rapid methods which would be sufficiently accurate for these purposes. In the second paper of this series (12), we reported on an improved refractometric method for the estimation of oil content and gave the results of some preliminary studies on the relation between refractive index and iodine value. It is the purpose of the present paper to present the experimental work that led to the development of a rapid refractometric method for the estimation of iodine value.

A review of the literature reveals that, while it has been commonly recognized that a relation exists between certain physical and chemical constants of fatty oils and fats, it is only recently that any attempt has been made to utilize the correlation between refractive index and iodine value as a basis for a rapid analytical procedure. Lewkowitsch (15) classified the physical and chemical constants of a large number of oils, fats and waxes but failed to find a definite relation between refractive index and iodine value; this is contrary to the findings of Arnold (2) and Backer (4) who showed that iodine value, saponification number and refractive index were correlated. Niegemann and Kayser (17) reported that the iodine value of linseed oil may be approximated from a knowledge of the refractive index if the source of the oil is known. Lund (16), Pickering and Cowlshaw (19) and others have derived equations expressing the mathematical relations between refractive index and the chemical constants of fatty oils and fats. In the instance of freshly prepared linseed, soya bean, cottonseed and peanut oils, which have similar saponification numbers and low free fatty acid content, the latter workers found the simple equation: $-n_D^{40} = 1.4515 + 0.000117$ (iodine value) to hold. They studied the effect on refractive index of variations in free fatty

acid content, molecular weight, oxidized and hydroxylated acids and developed the equation—

$$n_D^{40} = 1.4643 - 0.000058 (\text{saponification value}) - \frac{0.0096 \text{ acid value}}{\text{saponification number}} + 0.000117 (\text{iodine value})$$

—which applied to linseed, soya bean, cottonseed, peanut, rapeseed, palm nut and cocoanut oils. It was pointed out that this formula only held for freshly prepared oils from sound material, as the refractive index increases with age owing to the fact that the decrease with increasing acidity is smaller than the increase caused by oxidation and polymerization.

From theoretical considerations, Wolff (21) confirmed Lund's experimentally developed relations and derived the expression: $n = 1 + d (0.5557 - 0.00022s + 0.000035I)$ where n = refractive index, d = density, s = acid number, and I = iodine value, the formula giving " n " values within 0.0003 units of those found experimentally. This formula holds good for the glycerides of the aliphatic series, but not for the hydroxy or cyclic acids.

Sudborough, Watson and Athawale (20) investigated the refractive index and iodine number of hydrogenated oils and found the relation between these two variables to be independent of the hydrogenation time and type of catalyst and to be virtually identical for cottonseed, linseed, peanut, mohua, sesame and sardine oils. The equation $n_D^{60} = 1.4468 + 1.03 \times 10^{-4} (\text{iodine value}) + 7.3 \times 10^{-3} (\text{iodine value})^2$ quite accurately represented the relations found.

Lathrap (14), reporting on perilla oil, stressed the close relation between refractive index and iodine value.

Pickard (18) stated that the refractive index of linseed oil varies with the source of the oil and, on pure untreated raw oils, increases with specific gravity and iodine value.

In the second paper of this series (12), we reported a correlation of 0.647 between the refractive index and iodine value of linseed oils extracted by diethyl ether. It was mentioned that the refractive index of ethyl ether extracts appeared to be sensitive to variations in the temperature at which the extracts were prepared and that these did not appear to be accompanied by corresponding changes in iodine value. It was suggested that a closer association between these variables might be obtained in expressed oils.

Recently, Zeleny and Coleman (22, 23) and Hopper and Nesbitt (13) have also described refractometric methods for estimating iodine value, based on the correlation between refractive index and iodine number. Zeleny and Coleman cited the correlation coefficient of 0.647 between the refractive index and iodine number of oils extracted with diethyl ether, reported in the second paper of our series, but overlooked, apparently, the report by Geddes (10) that he had found a correlation of 0.95 between the refractive index and iodine number of cold pressed oil.

Experimental

As the work of Lund (16) and Pickering and Cowlishaw (19) has shown that the relation between refractive index and iodine value is influenced by free fatty acids, oxidation and polymerization, only sound samples were employed in this study. They represented commercial carlots of various grades of Western Canadian flaxseed marketed in 1934 and 1935, and several varieties and hybrids submitted by Canadian plant breeders for quality tests. In view of the fact that the authors (12) had previously secured a relatively low correlation between the refractive index and iodine value of linseed oils extracted by diethyl ether, a comparative study of these constants and their interrelations was made on ether-extracted and cold pressed linseed oils from 110 samples of flaxseed. The samples were ground to a fine pulp in a Hobart model 6-burr mill (equipped with stationary burr No. 4317, No. 2 R.N. Sta. MCH No. 6 Ex P.G. and rotary burr No. 4318, No. 2 P.H. Rot. MCH No. 6 Ex P.G.) with a setting of from 5.0 to 5.5.

The ethyl ether extracts were prepared by extracting a 10-gm. sample of the flax pulp, which had been dried overnight *in vacuo* at 98 to 100° C., with anhydrous alcohol-free and peroxide-free ethyl ether for 16 hr. in a Soxhlet extractor using Whatman double-thickness thimbles and a siphoning rate of one per minute. In order to remove traces of starch, the extracts were filtered through a sintered glass filter and transferred directly to a 125 cc. Erlenmeyer flask, the extraction flask being washed three times with fresh solvent. The excess ether was distilled off on a water bath maintained at 70° C. and the extract dried *in vacuo* for 3 hr. at 98 to 100° C. at a pressure not exceeding 25 mm. mercury. The entire technique was carefully standardized in order to avoid differences in treatment.

The expressed oils were prepared by pressing 25 gm. of the ground samples, at room temperature, in a special $2\frac{1}{4}$ -in. cylinder at a pressure of approximately 16,000 lb. per sq. in. in the Carver laboratory hydraulic press. A filter pad of Whatman No. 4 paper placed at the bottom of the press cylinder obviated the necessity of filtering the expressed oil. Sufficient oil for the refractometric and iodine value determinations was obtained after five to ten minutes' pressing; this was collected in small glass vials and the above tests carried out on the same day. Preliminary experiments revealed that variations in pressure from 1,000 to 20,000 lb. per sq. in. and in pressing time from 3 min. to 1 hr. at room temperature were without influence on the refractive index or iodine value of the expressed oil. It was found, however, that a slight increase in refractive index resulted when the hot plates between which the press cylinder is placed, were maintained at temperatures in excess of approximately 70° C.

The refractive indices of the oils were determined at 25° C. with a Zeiss refractometer equipped with interchangeable water-jacketed prism heads, as described in a previous paper (12); the "linseed oil" prism with arbitrary scale values of -5.0 to 105.0 corresponds to refractive indices of from 1.48986 to 1.46514.

Iodine values were determined in duplicate by the Wijs method according to the official A.O.A.C. (3) method, chloroform being used as the solvent; this method calls for a sample weight of 0.1 to 0.2 gm. oil and a contact time of 30 min. in the dark. There is a considerable lack of uniformity in the specifications given by different authorities for conducting the Wijs method and several studies have shown that variations in excess reagent, time and temperature of reaction affect the iodine value. The specifications of the American Society for Testing Materials (1) correspond closely to those of the A.O.A.C.; on the other hand, the Federal Specifications Board (7) specify the use of an 0.09 to 0.15 gm. sample and a reaction time of 1 hr. at 21 to 23° C. for raw linseed oil.

The refractometric and iodine values for the extracted and expressed oils prepared from the 110 samples of flaxseed were submitted to statistical analysis, the results of which are summarized in Table I.

TABLE I

STATISTICAL CONSTANTS FOR DATA ON REFRACTOMETRIC SCALE READING AND IODINE VALUE OF ETHER EXTRACTED AND COLD PRESSED OILS PREPARED FROM 112 SAMPLES OF FLAXSEED

	Mean	Min.	Max.	S.D.	C.V.
Refractometric scale reading at 25° C.*					
Ethyl ether extract (a)	46.63	37.4	60.0	5.36	11.49
Cold pressed oil (b)	48.37	37.6	62.3	5.87	12.14
Iodine value (Wijs)					
Ethyl ether extract (x)	176.66	151.2	196.0	10.71	6.06
Cold pressed oil (y)	178.88	152.6	200.7	11.02	6.16

SIMPLE CORRELATION COEFFICIENTS

	<i>r</i>	5% point
<i>r_{ab}</i>	0.9498	0.195
<i>r_{ax}</i>	— .9188	.195
<i>r_{by}</i>	— .9781	.195
<i>r_{xy}</i>	.9529	.195
<i>r_{ay}</i>	— .9392	.195

REGRESSION EQUATIONS AND STANDARD ERRORS OF PREDICTION

Iodine value (ethyl ether extract) on refractometric scale reading ethyl ether extract:—
 $x = 262.29 - 1.8364a$. Standard error of prediction, 4.18.

Iodine value (cold pressed oil) on refractometric scale reading (cold pressed oil):—
 $y = 267.64 - 1.8351b$. Standard error of prediction, 2.31.

(*) The refractive indices corresponding to the tabulated scale readings are as follows:—

	Mean	Min.	Max.
Ethyl ether extract	1.47890	1.48094	1.47587
Cold pressed oil	1.47851	1.48090	1.47534

For simplicity and convenience, the calculations were made on the refractometer scale readings without conversion to refractive indices; and in interpreting the statistics, it should be borne in mind that scale reading is inversely related to refractive index. The mean refractive index of cold pressed oil is lower and the mean iodine value higher than the corresponding values for the ether-extracted oil; as the refractive index and iodine value of both the extracted and expressed oils are positively correlated, this implies that the two oils are not strictly comparable in character. The correlation of 0.9781 between these variables for the cold pressed oils is significantly higher than the corresponding statistic 0.9188 for the ether-extracted oils; moreover, the correlation 0.9392 between the refractive index of ether-extracted oil and the iodine value of the cold pressed oil is significantly lower than the above value 0.9781, which indicates that the refractive index of the ether-extracted oil has been altered in an irregular manner by the processes involved in its preparation. As will be shown later, this may be ascribed to the sensitivity of the refractive index of linseed oil to unavoidable variations in the heat treatment to which it is subjected. It is of interest to note, however, that the correlation 0.9188 between refractive index and iodine value for the ethyl ether extracts is considerably higher than that previously reported by the authors (12), namely, 0.647; this is presumably due to the special precautions taken to standardize the technique employed in the present study for the preparation of the extracts. In evaluating different lots of flaxseed, it is desirable to obtain the oil in an essentially unchanged condition and this is most closely approached by cold pressing. This method is also preferable from the standpoint of cost, convenience and time and the greater accuracy with which iodine value may be predicted.

TABLE II
STATISTICAL CONSTANTS FOR REFRACTOMETRIC SCALE
READING AND IODINE VALUE OF COLD PRESSED OILS
PREPARED FROM 339 SAMPLES OF FLAXSEED

Statistic	Refractometric* scale reading, 25° C. (b)	Iodine value (Wij's) (y)
Mean	44.77	185.68
Minimum	37.0	152.6
Maximum	62.3	201.7
S. D.	5.64	10.38
C. V.	12.62	5.54

Correlation—Scale reading x iodine value $r_{by} = -0.980$.

Regression of iodine value on refractive index $y = 266.18 - 1.7980b$.

Standard error of prediction of iodine value = 2.08.

* The refractive indices (n_D^{25}) corresponding to the mean, minimum and maximum scale readings are 1.47931, 1.48125 and 1.47534 respectively.

In view of these considerations, the cold pressed oils from an additional 227 samples were analyzed in order to secure a more reliable basis for establishing the conversion equation for the estimation of iodine value from the refractometric scale readings. The results for the entire series of 339 samples are summarized in Table II. The correlation obtained between scale reading

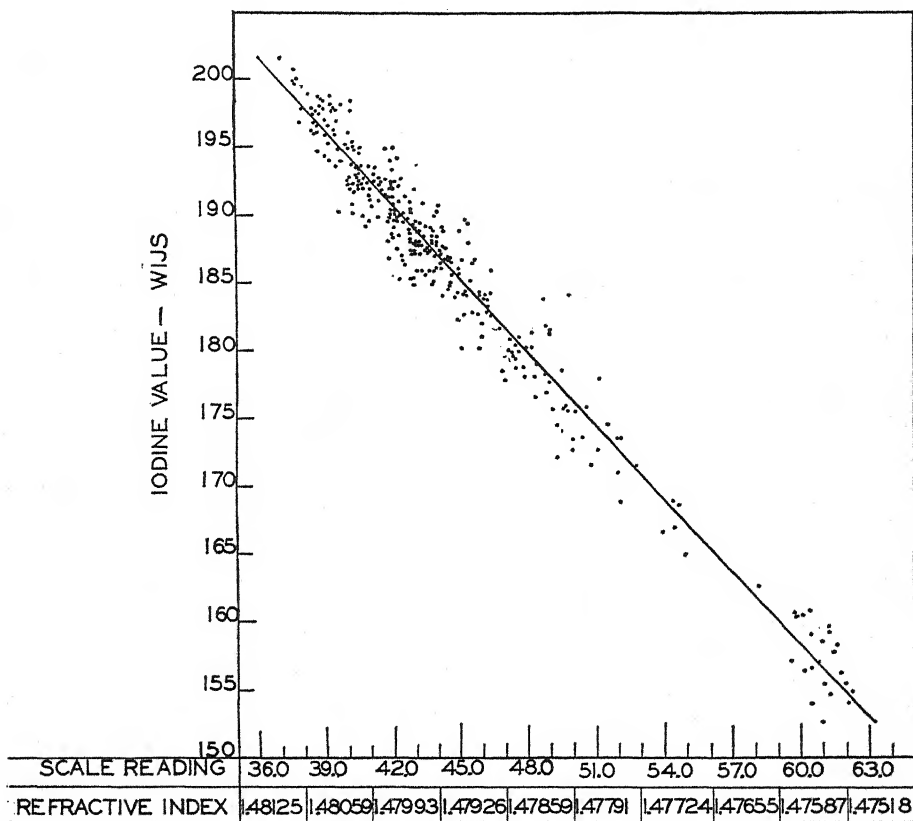


FIG. 1. Regression of iodine value on Zeiss refractometric scale reading for cold pressed linseed oils.

and iodine value of -0.980 permits prediction of iodine value from the regression equation with a standard error of prediction of 2.1 units, as compared with a correlation of -0.919 and a standard error of 4.2 units for the ether-extracted oils from 112 samples. The relation between these variables is illustrated graphically in Figs. 1 and 2 for the expressed and extracted oils respectively, and a conversion chart for estimating iodine values from the refractive indices of cold pressed oils is given in Table III.

As an index of the reliability of the refractometric method for estimating iodine value, it is of interest to compare these data with those recently published by Zeleny and Coleman (22, 23) and Hopper and Nesbitt (13).

TABLE III

CONVERSION CHART FOR COMPUTING WIJS IODINE VALUE OF COLD PRESSED OIL FROM ZEISS REFRACTOMETER SCALE READING AND CORRESPONDING REFRACTIVE INDEX*

Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)	Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)	Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)	Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)
1.48147	35.0	203.2	1.48048	39.5	195.2	1.47950	43.9	187.2	1.47852	48.3	179.3
1.48145	35.1	203.1	1.48046	39.6	195.0	1.47948	44.0	187.1	1.47850	48.4	179.2
1.48143	35.2	202.9	1.48044	39.7	194.8	1.47946	44.1	186.9	1.47848	48.5	179.0
1.48140	35.3	202.7	1.48041	39.8	194.6	1.47944	44.2	186.7	1.47845	48.6	178.8
1.48138	35.4	202.5	1.48039	39.9	194.4	1.47941	44.3	186.5	1.47843	48.7	178.6
1.48136	35.5	202.4	1.48037	40.0	194.3	1.47939	44.4	186.3	1.47841	48.8	178.4
1.48134	35.6	202.2	1.48035	40.1	194.1	1.47937	44.5	186.2	1.47838	48.9	178.3
1.48132	35.7	202.0	1.48033	40.2	193.9	1.47935	44.6	186.0	1.47836	49.0	178.1
1.48129	35.8	201.8	1.48030	40.3	193.7	1.47933	44.7	185.8	1.47834	49.1	177.9
1.48127	35.9	201.6	1.48028	40.4	193.5	1.47930	44.8	185.6	1.47832	49.2	177.7
1.48125	36.0	201.5	1.48026	40.5	193.4	1.47928	44.9	185.4	1.47829	49.3	177.5
1.48123	36.1	201.3	1.48024	40.6	193.2	1.47926	45.0	185.3	1.47827	49.4	177.4
1.48121	36.2	201.1	1.48022	40.7	193.0	1.47924	45.1	185.1	1.47825	49.5	177.2
1.48118	36.3	200.9	1.48019	40.8	192.8	1.47922	45.2	184.9	1.47823	49.6	177.0
1.48116	36.4	200.7	1.48017	40.9	192.6	1.47919	45.3	184.7	1.47821	49.7	176.8
1.48114	36.5	200.6	1.48015	41.0	192.5	1.47917	45.4	184.6	1.47818	49.8	176.6
1.48112	36.6	200.4	1.48013	41.1	192.3	1.47915	45.5	184.4	1.47816	49.9	176.5
1.48110	36.7	200.2	1.48011	41.2	192.1	1.47913	45.6	184.2	1.47814	50.0	176.3
1.48107	36.8	200.0	1.48008	41.3	191.9	1.47911	45.7	184.0	1.47812	50.1	176.1
1.48105	36.9	199.8	1.48006	41.4	191.7	1.47908	45.8	183.8	1.47809	50.2	175.9
1.48103	37.0	199.7	1.48004	41.5	191.6	1.47906	45.9	183.7	1.47807	50.3	175.7
1.48101	37.1	199.5	1.48002	41.6	191.4	1.47904	46.0	183.5	1.47805	50.4	175.6
1.48099	37.2	199.3	1.48000	41.7	191.2	1.47902	46.1	183.3	1.47803	50.5	175.4
1.48096	37.3	199.1	1.47997	41.8	191.0	1.47899	46.2	183.1	1.47800	50.6	175.2
1.48094	37.4	198.9	1.47995	41.9	190.8	1.47897	46.3	182.9	1.47798	50.7	175.0

* The values were computed from the regression equation: Iodine value = $266.18 - 1.798$ (Zeiss scale reading) at 25° C.

TABLE III—*Concluded*

CONVERSION CHART FOR COMPUTING WIJS IODINE VALUE OF COLD PRESSED OIL FROM ZEISS REFRACTOMETER SCALE READING AND CORRESPONDING REFRACTIVE INDEX*

Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)	Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)	Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)	Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)
1.48092	37.5	198.8	1.47993	42.0	190.7	1.47895	46.4	182.8	1.47796	50.8	174.8
1.48090	37.6	198.6	1.47991	42.1	190.5	1.47893	46.5	182.6	1.47793	50.9	174.7
1.48088	37.7	198.4	1.47988	42.2	190.3	1.47890	46.6	182.4	1.47791	51.0	174.5
1.48085	37.8	198.2	1.47986	42.3	190.1	1.47888	46.7	182.2	1.47789	51.1	174.3
1.48083	37.9	198.0	1.47984	42.4	189.9	1.47886	46.8	182.0	1.47787	51.2	174.1
1.48081	38.0	197.9	1.47982	42.5	189.8	1.47883	46.9	181.9	1.47784	51.3	173.9
1.48079	38.1	197.7	1.47979	42.6	189.6	1.47881	47.0	181.7	1.47782	51.4	173.8
1.48077	38.2	197.5	1.47977	42.7	189.4	1.47879	47.1	181.5	1.47780	51.5	173.6
1.48074	38.3	197.3	1.47975	42.8	189.2	1.47877	47.2	181.3	1.47778	51.6	173.4
1.48072	38.4	197.1	1.47972	42.9	189.0	1.47874	47.3	181.1	1.47776	51.7	173.2
1.48070	38.5	197.0	1.47970	43.0	188.9	1.47872	47.4	181.0	1.47773	51.8	173.0
1.48068	38.6	196.8	1.47968	43.1	188.7	1.47870	47.5	180.8	1.47771	51.9	172.9
1.48066	38.7	196.6	1.47966	43.2	188.5	1.47868	47.6	180.6	1.47769	52.0	172.7
1.48063	38.8	196.4	1.47963	43.3	188.3	1.47866	47.7	180.4	1.47767	52.1	172.5
1.48061	38.9	196.2	1.47961	43.4	188.1	1.47863	47.8	180.2	1.47764	52.2	172.3
1.48059	39.0	196.1	1.47959	43.5	188.0	1.47861	47.9	180.1	1.47762	52.3	172.1
1.48057	39.1	195.9	1.47957	43.6	187.8	1.47859	48.0	179.9	1.47760	52.4	172.0
1.48055	39.2	195.7	1.47955	43.7	187.6	1.47857	48.1	179.7	1.47758	52.5	171.8
1.48052	39.3	195.5	1.47952	43.8	187.4	1.47854	48.2	179.5	1.47755	52.6	171.6
1.48050	39.4	195.3									

* The values were computed from the regression equation: Iodine value = $266.18 - 1.798$ (Zeiss scale reading) at 25° C.

The experimental technique and the results obtained by the three laboratories are summarized in Table IV and the agreement obtained indicates that the refractometric method of estimating iodine value is quite reliable, especially when it is considered that the methods employed for preparing the oils and determining their iodine values were not identical.

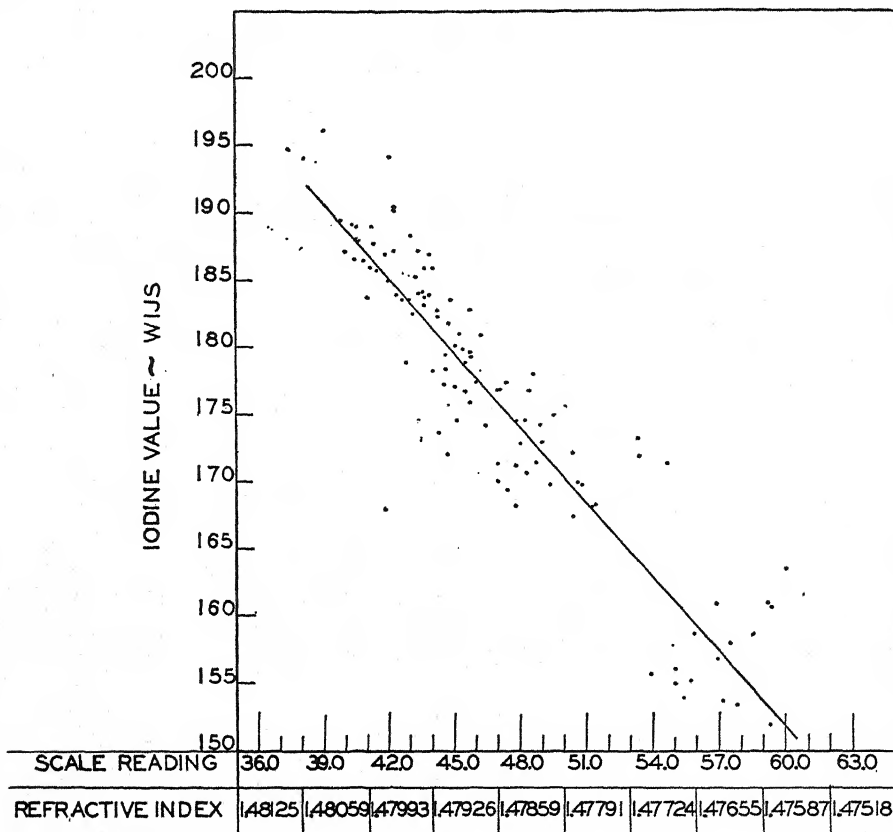


FIG. 2. Regression of iodine value on Zeiss refractometric scale reading for ethyl ether extracted linseed oils.

Zeleny and Coleman (22) prepared the majority of their oil samples by a partial extraction of the ground samples with petroleum ether, the extract being dried in an air oven at 105° C. for 30 min., and they present data for six samples showing that the refractive indices of oils prepared by this procedure are practically identical with those obtained by cold pressing. On the other hand, in order to conserve time and thereby render the method more applicable to routine testing, Hopper and Nesbitt (13) heat the meal in a beaker suspended in a steam bath for 20 min. and express the oil between hot plates at 60 to 70° C. They present comparative refractive indices and iodine values of oils expressed by this method with those obtained by cold

TABLE IV

COMPARISON OF THE RELATIONS BETWEEN REFRACTIVE INDEX OF RAW LINSEED OIL AND IODINE VALUE AS REPORTED BY DIFFERENT WORKERS.

—	Zeleny and Coleman	Hopper and Nesbitt	Lehberg and Geddes
Method of preparing oil	Either by cold pressing in a laboratory hydraulic press at 16,000 pounds per square inch or by partial extraction with petroleum ether, and drying extract in air oven at 105° C. for 30 minutes.	Meal heated in a steam bath for 20 minutes and warm pressed between hot plates at 60 to 70° C.	Oil cold pressed from ground meal at 16,000 pounds per square inch.
Method of determining refractive index	Zeiss refractometer at 25° C.	Abbé refractometer at 25° C.	Zeiss refractometer at 25° C.
Method of determining iodine value	Wijs method according to instructions of Federal Specifications Board (reaction time 60 minutes).	Wijs method according to "official" procedure (presumably A.O.A.C. specifications) but using a reaction time of 60 minutes.	Wijs method according to A.O.A.C. specifications (reaction time 30 minutes).
Number of samples analyzed	96	1485	339
Refractive index range	1.47589 to 1.48065	1.47420 to 1.48047	1.47534 to 1.48125
Iodine value range	155.4 to 197.3	144 to 196	152.6 to 201.7
Correlation to refractive index α iodine value	0.996	0.989	0.980
Regression equation	Iodine value = $8584.97 (n_D^{25}) - 12513.83$	$n_D^{25} = 1.45723 + .00011846$ (iodine value)	Iodine value = $266.18 - 1.7980$ (Zeiss scale reading).
Standard error of estimate of iodine value from refractive index	0.8	1.5	2.1

COMPARISON OF CONVERSION CHARTS

Refractive index n_D^{25}	Estimated iodine value (Wijs)		
	Zeleny and Coleman	Hopper and Nesbitt	Lehberg and Geddes
1.4750	149.0	149.4	151.5
1.4755	153.3	154.2	155.4
1.4760	157.6	158.4	159.4
1.4770	166.2	166.9	167.3
1.4780	174.8	175.3	175.2
1.4790	183.3	183.8	183.1
1.4800	191.9	192.2	191.2
1.4810	200.5	200.7	199.5
1.4817	206.5	206.6	205.0

pressing on 26 samples of flaxseed. The mean iodine value of the warm pressed oils was 0.1 units higher and the mean refractive index 0.00007 units higher than for the cold pressed oils, and they conclude that in the majority of the samples the differences are within the limits of reasonable experimental error. On casual examination of their results, we noted that the refractive index of the warm pressed oils exceeded that of the cold pressed in 18 out of 26 cases, the differences ranging as high as 0.00026 units. Accordingly, a statistical analysis of their data was made which showed that, while the mean iodine values of cold and warm pressed oils were not significantly different, the refractive index of the warm pressed oils was significantly higher.

As mentioned earlier in this paper, the writers also found the refractive index of the oil to be increased slightly when the oil was prepared by pressing between hot plates maintained above approximately 70° C.; this would account for the slightly higher predicted iodine values reported by the other workers.

That the use of heat in preparing the oils is undesirable is substantiated by experiments we have conducted in which cold pressed linseed oil has been heat-treated for 30 min. in the presence of air at 25° C. increments of temperature from 25° to 200° C. The refractive indices began to increase at approximately 75° C., while the iodine values remained substantially unaltered until a temperature of approximately 125° C. was attained.

These results serve to explain the closer degree of association between refractive index and iodine value of cold pressed as compared with ether-extracted oils found by the writers, and clearly indicate the desirability of preparing the oils by cold pressing, as this procedure is the least likely to induce changes in physical and chemical characteristics.

Discussion

The rapid optical procedure for estimating iodine value, in conjunction with the improved refractometric method previously described by the authors, has been employed in flaxseed studies carried out by this laboratory since January, 1936, and has been found to be of great utility in conducting surveys of the quality of flaxseed produced in different districts of Western Canada and in evaluating new varieties and hybrids submitted by plant breeders, where the greatest accuracy is not required. The method is not applicable to flax that is musty or heated, although it has been found to give reliable results with immature, frost damaged or scabby flax. Zeleny and Coleman (22, 23) have made similar observations in this connection and point out that the method is intended to apply only to samples of oil from freshly ground unaltered material. The refractometric method obviously cannot be employed on commercially prepared linseed oils but should be of considerable utility to linseed crushers in securing a measure of the intrinsic drying values of their raw material.

Acknowledgment

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INHERITANCE OF QUALITY AND QUANTITY OF OIL IN FLAX IN RELATION TO OTHER PLANT CHARACTERS¹

By W. G. MCGREGOR²

Abstract

Data from 21 varieties of flax, grown at the Central Experimental Farm over a five-year period, were analyzed statistically to determine the relation of quantity and quality of oil to seed size, days to maturity, days from flowering to maturity, and height of plant. In addition, hybrids of Cyprus \times Ottawa 770B and Buda \times Ottawa 770B were studied to determine the genetic basis for the inheritance of quality of oil, flower type, color of seed, color of oil, seed size and height of plant.

The refractometric method for determining the quantity and quality of oil was compared with the ether extraction method for oil content and the Wijs method for iodine number and found to be very practical for breeding studies.

In the variety test, high oil content was associated with a long period from blossoming to maturity and with large-seeded varieties.

In the hybrids, iodine number, seed size, and height of plant are apparently dependent on several genetic factors. No significant association between oil content, iodine number, seed size or height of plant was found among these hybrids. The inheritance of flower and seed type has been explained on the basis of a single factor, the Ottawa 770B type with white, narrow, involute petal and greenish-yellow seed being inherited as a simple recessive or the expression of several very closely linked recessive genes. An association of high iodine number with this factor for yellow seed color was indicated in both hybrids.

Although insufficient data were collected to give definite conclusions, evidence indicated that color of oil, as measured by carotinoid pigment content, had a genetic basis. No correlation was indicated between carotene pigment content and the quantity and quality of the oil or color of the seed.

Introduction

Heretofore, improvement in flax has been based largely on yield and on disease resistance, especially resistance to wilt. Reasonable advances in attaining these qualities have already been accomplished so that the next problem is to improve the yield of oil and its drying qualities. Flaxseed is purchased primarily for the oil it contains, which represents from two-thirds to three-fourths of the value of the manufactured products. Methods of oil determination have already been established which, if adopted for commercial purposes, will put a premium on the quantity and quality of the oil present in any market sample. A recognition of quality on the official inspection certificate would be an added incentive to the farmer and would create a demand for varieties of high quality.

Yellow-seeded varieties of flax have been observed to be high in quality. A variety of the yellow-seeded type would appear to have other advantages over the more common brown-seeded varieties, *viz.*, in the production of a lighter colored oil cake and especially a lighter colored oil. In a flax improvement program it is necessary to have a knowledge of the manner in which these characters are inherited and their relation to one another.

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*Studies in Flax***Literature Review**

Inheritance of characters in flax has been previously studied and reviewed by Tammes (21, 22) and Shaw, Khan and Alam (20).

Many early investigators have made observations on the relation of various kernel characters to the chemical composition of flaxseed. Eyre and Fisher (8), Birchard (1), Rabak (19) and Geddes and Lehberg (10) observed that in practically all cases an increase in oil content accompanied an increase in size of the seed. Coleman and Fellows (4) in a more extensive study considered test weight per bushel, color and size of seed. Oil content was found to increase gradually as the kernels grew larger. It was pointed out, however, that this relation is not specific and that small plump seeds often contain as great a percentage of oil as a sample containing very large kernels. No relation was found between depth of seed color and oil content.

Johnson (14), in a statistical study, found significant simple correlations between the oil content and weight per 1000 kernels, date of maturity and number of days from full bloom to maturity. A high multiple correlation between percentage of oil and the above variables was taken to indicate that the total effect of these characters determines to a considerable extent the percentage of oil. Significant inter-annual correlations of oil content, seed weight, days to maturity and the period from full bloom to maturity indicated that these characters are relatively constant in their inheritance. A low multiple correlation of iodine number with these characters was interpreted as suggesting that iodine number was inherited independently of the plant and seed characters studied.

Other investigators have not agreed with these findings on the relation of size of seed to oil content. Leather (16) analyzed samples of flaxseed collected from several provinces in India and concluded there was no relation between size of seed and oil content. More recently Ermakov (6) used a technique in which individual seeds were analyzed. The correlations observed were occasionally negative, and in seeds giving the same oil content one was often twice the weight of the other and *vice versa*. These differences were ascribed to the genetical nature of the different seeds.

The iodine number, as an index of drying quality, has been studied in relation to oil content and certain other characters. Johnson (14) found a significant negative correlation of -0.309 between iodine number and weight per 1000 seeds. Dillman (5) has reported the work of Arny who explained the inheritance on the action of a single factor with low iodine number dominant. Yellow seed-coat appeared to be linked with high or medium iodine number.

Related Studies in Other Oil Bearing Seeds

A study of the chemical composition of corn was begun in 1896 at the Illinois Agricultural Experiment Station (25). After 28 years of continuous selection for high and low oil content, and high and low protein content, four types were produced which were distinctly different in composition. Selection was more effective in bringing a change in oil content than in protein content.

It was suggested that the primary materials from which fat is manufactured, namely, carbon dioxide and water, are usually furnished to the plant in unlimited supply, while the formation of protein is essentially dependent upon the supply of available nitrogen in the soil.

An experiment similar to that with corn was started with soybeans by Woodworth (26) in 1914. While selection within pure lines was ineffective either in the high or low direction, the F_1 hybrids between high and low lines were intermediate and the F_2 showed transgressive segregation. No attempt was made to estimate the number of genes which were responsible for the difference between the high and low strains.

An experiment was started at the Wisconsin Station in 1912 by Cole, Lindstrom and Woodworth (3) to determine whether any progress could be made in selection of soybeans for quality of the oil. After seven years' selection the quality and quantity of oil showed a high degree of variability but no consistent relation. Indirect evidence and some experimental data indicated that high quality was correlated with late maturity. Their data indicate that genotypes were isolated for type of oil and other characteristics.

Recently increased interest has been shown in the oil content of sunflowers, ground nut, and numerous other plants which have possibilities in the production of oil. Ermakov (6) states that Hildebrandt has obtained a correlation as high as 0.66 ± 0.07 between oil content and color of seed in sesamum. Ermakov found it possible to eliminate lines of low oil content in sunflowers with improved methods of technique but observed no relation between color of seed and oil content. More recently Ermakov *et al.* (7) have studied the intergeneric and interspecific differences in oil content of lupine seeds. A wide variation in oil content was found in this material.

Method of Procedure

Since scarcely any information was available regarding the oil content and quality of flax varieties grown in Canada, a survey of the varieties grown at the Central Experimental Farm, Ottawa, Canada, was made during the years 1929 and 1931-1934 inclusive. A number of foreign and domestic linseed varieties including fibre types were considered in this test. They were grown in a quadruplicate row-row test arranged systematically, and all plots of each variety were bulked for analysis of oil content and iodine number.

Characters studied in relation to oil content and iodine number included height of plant, weight per 1000 kernels, weight per measured bushel, days to maturity, length of period from full bloom to maturity, and yield. Analyses of variance and correlations with these characters for the 21 varieties over the five-year period were made in accordance with methods outlined by Goulden (13).

Hybridization studies were begun in 1933. Varieties from which some data on the oil content and iodine number were available, as ascertained from the varietal survey, were used. Although several combinations were made,

only two, Ottawa 770B \times Cyprus and Ottawa 770B \times Buda, have been studied in detail. The description of these varieties follows:—

Cyprus C.A.N. 2110* is short-strawed with a blue, rather flat flower, and medium broad petals. The anthers, filament, stigma and style are blue. The seed is brown and very large, averaging 9.2 gm. per 1000 kernels at this station. In maturity it is medium, blossoming very early and having a long period from blossoming to maturity. The oil content over a five-year period averaged $39.5 \pm 1.94\%$ and the iodine number 170.0 ± 7.56 .

Buda C.A.N. 2104 has a smaller, somewhat lighter blue, tubular to funnel-shaped flower. The anthers are blue with light blue filaments. The style is deep blue with a mauve to purple stigma. The seeds are brown and very small, averaging 4.1 gm. per 1000 kernels. The oil content over a five-year period averaged $38.1 \pm 1.71\%$ and the iodine number 186.2 ± 4.17 .

Ottawa 770B, C.A.N. 2152, has a white flower with a narrow involute petal. The anthers are yellow, the filament and stigma are white, the style being light yellow at the base and white at the tip. The seeds are greenish-yellow, of medium size, averaging 6.0 gm. per 1000 kernels. In maturity it is very late. The oil content averaged $41.2 \pm 0.80\%$ and the iodine number 187.7 ± 5.34 .

These hybrids were made in the field during the summer of 1933 and the F_1 generation was grown in the greenhouse during the winter of 1933–34. The F_2 was grown in the field in 1934. Individual F_2 plants were tagged and notes taken on the shape and color of the floral parts. The plants were pulled when mature and taken to the laboratory where each plant was examined as to height and color of seed. The third generation was grown in the field during the season of 1935. Forty-eight seeds of each F_2 line chosen were seeded in two rows, each four feet long. Fifty lines of each parent were seeded in a similar manner in alternate plots. The F_3 lines were pulled and dried in the drying barn after which they were examined and threshed. Each sample was handpicked before taking weight per 1000 kernels and making an oil analysis. Weight per 1000 kernels was determined by counting 250 seeds at random in duplicate and weighing to ± 0.1 gm.

Oil determinations were made by both the ethyl ether extraction method and the improved refractometric method as described by Geddes and Lehberg (11).

A refractometric method for measuring quality, developed by the Grain Research Laboratory, was also used. A sample of ground, undried flax pulp was placed in the pressing cylinder of a Carver laboratory press and subjected to a pressure of approximately 16,000 lb. to the square inch for 10–15 min. and the refractive index of the oil determined. The iodine number was also obtained by the Wijs method.

Studies have shown a very close relation between the refractive index of the cold pressed, untreated, linseed oil and the Wijs iodine value. Data from

* C.A.N. = Canadian Accession Number.

the Grain Research Laboratory on a large number of samples covering many different varieties and types of flax have given a correlation coefficient of 0.980. In the work on this problem, when only 25 samples of each parent were analyzed, the correlation coefficient in each case was highly significant. Owing to the time required for making quality tests by the Wijs iodine method, the refractive index only was run on samples of Buda and the hybrid Buda X Ottawa 770B.

Color intensity was studied spectrophotometrically by a method used in the Grain Research Laboratory. This is a modification of the method for flour outlined by Geddes, Binnington and Whiteside (12). Four grams of undried flaxseed pulp, on a dry basis, was weighed into two 150 cc. glass-stoppered bottles to which were added, from a pipette, 100 cc. of a mixture of 93 parts by volume of a petroleum distillate, known commercially as varnish makers' naphtha, with seven parts by volume of absolute ethyl alcohol. The bottle was shaken for two hours in a mechanical shaker and the supernatant liquid decanted into 100-cc. lipless centrifuge tubes, closed with a rubber cap and centrifuged at approximately 2000 r.p.m. for 30 min. The clear extract was siphoned off with a capillary bore siphon, and the transmittancy determined in a 10 cm. cell at a wave-length of 435.8Å, a mercury arc being used as a light source. By this method the carotinoid and similar pigments are extracted. However, throughout this study they have been referred to as "carotene".

Survey of Varieties

Characters of a variety or strain, such as size of seed, height of plant, maturity and yield, might be expected to bear some relation to the quantity and quality of the oil produced. An analysis of variance of oil content, iodine number and size of seed, of the varieties and strains of flax that were tested throughout a five-year period is given in Table I. While these 21 lines include a fair sample of the leading varieties of flax, a period of five years is not as representative of the seasons as might be desirable. It will be noted in this table that there is a significant variation, as shown by the F value, for each character studied. Season as well as variety has a significant influence

TABLE I

ANALYSIS OF VARIANCE OF OIL CONTENT IN PER CENT, IODINE NUMBER AND WEIGHT PER THOUSAND KERNELS IN GRAMS

Source of variation	Degrees of freedom	Oil content		Iodine number		Size of seed		1% point
		Mean square	F value	Mean square	F value	Mean square	F value	
Total	104	4.331	4.6	46.19	3.8	1.68	9.7	1.47
Between years	4	25.655	27.1	540.34	44.9	2.40	13.9	3.56
Between varieties	20	13.604	14.4	890.09	74.0	75.91	43.9	2.03
Remainder	80	.947		12.03		.17		

on the oil content, iodine number and size of seed. The data on these characters for each year are given in the original thesis, but since space will not permit their publication here the average has been given in Table II.

TABLE II

AVERAGE DATA ON FLAX VARIETIES GROWN AT THE CENTRAL EXPERIMENTAL FARM FOR THE YEARS 1929 AND 1931-34 INCLUSIVE

Variety	Canadian Accession Number	Oil content, %	Iodine number	Wt. per 1000 K, gm.	Yield per acre, bu.	Days to maturity	Days from flowering to maturity	Length of straw, in.
Buda	2104	38.1	186.2	4.1	19.4	92.8	37.9	21.2
Chippewa	2105	38.8	179.0	4.8	18.2	95.7	39.3	22.4
Damask	2111	38.7	187.5	4.1	14.1	94.4	37.1	23.9
Diadem	2113	42.5	180.1	6.6	16.7	90.8	42.1	19.5
J. W. S.	2117	39.9	183.6	4.3	14.2	92.9	38.5	28.8
Linota	2120	38.6	188.5	4.4	16.9	94.0	39.7	23.2
N. D. R. 52	2130	38.8	184.6	4.3	18.7	93.1	39.3	23.2
N. D. R. 114	2131	38.9	183.2	4.9	17.4	91.0	35.6	22.9
Novelty	2135	41.9	178.5	5.4	18.7	94.8	41.5	22.3
Palermo	2136	41.3	179.9	7.1	18.1	98.0	45.2	22.2
Redwing	2140	40.1	184.6	4.7	19.5	93.3	40.4	22.0
Siberian	2142	40.8	182.3	4.4	19.0	95.6	39.3	22.0
Slope	2143	38.9	188.6	4.4	18.4	91.9	39.5	21.2
Slope 585	2144	39.3	183.8	4.7	18.0	92.4	38.3	22.3
Winona	2146	38.5	185.1	4.5	19.1	94.7	39.8	22.2
8B	2147	42.9	175.3	7.4	19.9	104.3	48.0	18.1
8C	2148	43.4	179.5	7.3	19.7	103.7	48.5	18.9
9B	2149	41.8	177.2	6.8	19.0	101.2	47.8	19.7
12C	2150	42.9	179.2	6.7	19.8	102.8	49.4	18.6
12C42	2151	41.5	174.7	7.1	18.3	104.8	51.4	19.8
Ottawa 770B	2152	41.2	187.7	5.5	16.8	102.8	43.3	22.1

The relation of quality or iodine number to the percentage of oil in the seed as measured by the correlation coefficient is shown in Table III. The high negative correlation between oil content and iodine number for varieties would indicate that varieties that yielded a good quantity of oil might be expected to yield oil of poor quality. However, the partial correlation of the fourth order, calculated from the correlations between varieties, considering size of seed, height, yield and days from full bloom to maturity, was -0.068 . This would indicate that were varieties considered that were similar in size, yield, height and days from full bloom to maturity, no relation would be found between oil content and iodine number. Johnson (14) reports an analogous relation between these characters in flax in which a simple negative correlation of -0.056 was changed to a positive partial correlation of 0.272 .

A highly significant positive correlation between oil content and size of seed is indicated, both for varieties and for years, in Table IV. In the case of iodine number, only the negative correlation for varieties is significant. Size of seed varies more with variety than with season, while oil content is influenced by season as well as variety. The partial correlation of the fourth order between size of seed and oil content is 0.259 . This may indicate that under conditions in which the varieties would have the same iodine

number, yield, height and days from flowering to maturity, no significant relation would be expected between oil content and seed size. Many investigators (1, 5, 8, 10, 14), dealing with varieties or commercial samples, have noted that in practically all cases an increase in oil content accompanied an increase in the size of seed.

TABLE III

CORRELATION OF IODINE NUMBER WITH OIL CONTENT, SIZE OF SEED, MATURITY AND HEIGHT OF PLANT

Source of variation	Degrees of freedom	Iodine number				
		Oil content	Size of seed	Days to maturity	Days flowering to maturity	Height of plant
Total	103	-0.391**	-0.445**	-0.485**	-0.422	0.377**
Between years	3	-.259	-.074	-.548	-.334	.663
Between varieties	19	-.717**	-.782**	-.622**	-.730**	.496*
Remainder	79	.172	.079	-.078	-.076	-.246*

* Significant at 5% level.

** Significant at 1% level.

The question of early maturity is one of the most important features of crop improvement in Canada. Notes were taken on both the number of days to maturity and the length of the period from full bloom to maturity. From the data presented in Table III, both days to maturity and days from flowering to maturity are negatively correlated with iodine number. Oil content, however, appears more closely associated with the length of the seed-forming period than the full length of the growing period. This agrees with data presented by Johnson (14). The correlations between years in days to maturity and days from flowering to maturity are highly significant in the instance of oil content but not of iodine number. Seasons in which the ripening period is extended have a very favorable effect on the oil content but do not appreciably affect iodine number. High iodine number is related to varieties which are slow in maturing.

TABLE IV

CORRELATION OF OIL CONTENT WITH SIZE OF SEED, MATURITY AND HEIGHT OF PLANT

Source of variation	Degrees of freedom	Oil content			
		Size of seed	Days of maturity	Days flowering to maturity	Height of plant
Total	103	0.742**	0.639**	0.757**	-0.393**
Between years	3	.963**	.918**	.958**	.237
Between varieties	19	.856**	.251	.874**	-.567**
Remainder	79	.135	.042	.130	-.267*

* Significant at 5% level.

** Significant at 1% level.

The varieties investigated were grown in a replicated yield test each season. No relation is indicated between yield per acre and oil content either between varieties or between years. A negative correlation, -0.430 , appears between iodine number and yield for varieties. As the 5% point is 0.423 this association is not convincingly significant.

The 21 varieties under investigation included several short- and long-strawed types. The relation of length of straw to oil content and to iodine number are both indicated. A positive correlation is found between height and iodine number but this is not highly significant. A more significant association is that between tall varieties and low oil content.

Multiple correlations for the percentage of oil and iodine number with the other variables such as size of seed, yield, height and days from flowering to maturity, indicate the extent to which their total effect has influenced either of these characters. The multiple correlation of oil content is 0.892 and highly significant. These variables would appear, therefore, to be of value in predicting oil content. The multiple correlation of iodine number with these variables, including oil content, is 0.825 and without oil content this correlation is 0.824 . This would indicate that while these variables exert a significant influence on iodine number, oil content adds little to their effect.

Inheritance Studies

Quality and Quantity of Oil

The parents used in this study varied in iodine number (Table V). Reciprocal crosses of Cyprus, a low quality variety, and Ottawa 770B, a medium high quality variety, gave F_3 progenies with iodine number ranging between the parents with few as high as Ottawa 770B and few as low as the higher lines of the Cyprus parent. The F_3 distribution in these crosses shows a slight but not significant skewness toward that of the high quality parent, the value of the g_1 statistic being -0.447 ± 0.288 .

In the hybrid 33-217, in which Ottawa 770B is crossed with Buda which has high quality, the F_3 progenies again show greater variability than the parents. The distribution approaches that of a normal curve with a value for g_1 of 0.180 ± 0.312 . Although a similar result might be obtained from environmental effects only, these hybrids were grown on a very small area and all lines may be expected to have been exposed to almost similar conditions. The distribution would suggest that quality is governed by several factors with no dominance. These data do not agree with those of Arny who, as reported by Dillman (5), has explained the inheritance of quality in linseed oil on the basis of a single factor with low quality dominant.

The oil content of Cyprus and Ottawa 770B did not differ as much as did iodine number. The Cyprus parent averaged $42.5 \pm 0.09\%$ of oil and the Ottawa 770B parent $41.5 \pm 0.09\%$. The oil content of the reciprocal hybrid F_3 progenies averaged 41.2 ± 0.14 and $41.9 \pm 0.11\%$ respectively.

TABLE V
FREQUENCY DISTRIBUTION OF IODINE NUMBER FOR PARENTS AND F_3 LINES

Parent or cross	No. lines	Classes for iodine number																		S.D.	C.V.	
		152.5	155.0	157.5	160.0	162.5	165.0	167.5	170.0	172.5	175.0	177.5	180.0	182.5	185.0	187.5	190.0	192.5	195.0			197.5
Cyprus	22	4	6	6	2	2	1														3.41	2.15
Ottawa 770B	25														7	6	10	2			2.16	1.14
Buda	24																	1	18	5	1.36	0.70
33-215																						
Ott. 770B × Cyprus	35					1	1		1	2	3	10	5	7	2	3					5.52	2.92
33-216																						
Cyprus × Ott. 770B	34						2	1	4	5	4	5	5	4	2	2					5.92	3.32
33-217																						
Ott. 770B × Buda	58													6	9	12	9	12	4	6	4.42	2.32

Flower and Seed Type

The characters of the flower and seed of the parents, as already described, differed greatly. The F_1 from the cross of Cyprus \times Ottawa 770B resembled the Cyprus parent. The segregation observed in the F_2 generation was 35 blue, Cyprus type; 10 white, Ottawa 770B type, and in the reciprocal 62 blue, Cyprus type; 16 white, Ottawa 770B type. The deviations from expected, on the basis of a major factor differentiating these two types, were 1 and 3 respectively which are well within the error expected. Twenty-one selections with white flower were grown in the F_3 generation and bred true for the Ottawa 770B type. Forty-eight selections from among the blue-flowered segregates were grown, 17 of which bred true and 31 segregated as in the F_2 generation.

In the cross Buda \times Ottawa 770B, the F_1 generation was similar in all respects to the Buda parent. The F_2 generation segregated as follows:— 259 blue, Buda type : 77 white, Ottawa 770B type; and the reciprocal 86 blue, Buda type : 28 white, Ottawa 770B type. The deviations in this cross are again well within the error to be expected on the basis of a monohybrid segregation. Thirty-nine selections with white flower bred true for flower type and, of 56 selections with blue flower, 18 bred true and 38 segregated as in the F_2 generation.

A deficiency of white progeny in the F_2 generation when crossing a white crimped-petal variety with blue flax has been reported by Tammes (21) and Myers (18). Tammes has attributed this deficiency to a semi-lethal effect of one of the basic factors for petal color. It has also been demonstrated by Kappert (15) that a white crimped-petal type may give a deficiency when crossed with certain blue types but not with others. In the F_2 of the crosses reported here, it will be noted that although the recessive class is smaller in each case than the theoretical, it is still well within the deviation expected on the basis of random sampling.

Yellow anthers and yellow seeds were always found associated with the white crimped-petal segregates while no blue-flowered segregates had the crimped petal. These results may be explained by assuming a basal factor which controls the color in the petal, anther, filament, style and stigma and also the color of the seed as well as the flatness or crimpiness of the petal, or several other closely linked factors. This situation has also been reported by Myers (18) in using Ottawa 770B in crosses with Redwing.

Color of Oil

The Ottawa 770B variety was used in this project because it combined high iodine value with yellow seed of very low carotene pigment content. The average of the lines tested from the 1935 crop was 11.6 ± 0.6 parts per million of pigment expressed as "carotene" while the Buda parent averaged 17.9 ± 0.6 p.p.m.

Slight variations in the depth of color of the yellow seeds were noticed among the lines selected but these differences were not so apparent among the brown types. During the pressing of these samples with the Carver

press, distinct differences were noticed in the color of the cold pressed oil. A number of samples were then analyzed for "carotene" pigment by the method already described. Samples differing by five parts per million yielded a cold pressed oil which showed differences in color readily discernible to the naked eye.

Light color of seed in flax has been shown by Tammes (21) to be due wholly or partly to the cotyledon showing through an entirely colorless seedcoat, the darker colors being due to pigment in the seedcoat. It was not surprising to find in the analysis of the hybrid lines, which have very distinct differences in the intensity of the yellow in the cotyledons, wide differences in pigment content, as measured by "carotene" pigment in parts per million. Selections among the yellow-seeded types from the cross Buda \times Ottawa 770B varied from 17.6 p.p.m. to 35.9 p.p.m. with an average of 24.3. The brown-seeded types varied over almost as wide a range as did the yellow-seeded types. While only small numbers of the progeny were studied in this manner the segregation obtained indicated that this character was probably dependent on several factors for its expression.

Seed Size

Frequency distributions of the weight per thousand kernels in grams are presented in Table VI and summarized in Table VII. While the reciprocal progenies from crossing Cyprus and Ottawa 770B show greater variability than Buda crossed with Ottawa 770B or Cyprus, these reciprocals do not differ appreciably in their means and standard deviations and have been combined in Table VI.

The greater variability of the F_3 progeny means compared with the parents indicates that segregation for size factors has taken place. Although a considerable number of the F_3 hybrid lines are within the range of the smaller parent, only in three instances has the larger parent been recovered in these progenies. The distribution curves for these progenies, when calculated on an arithmetic basis, approach very closely a normal curve. The g_1 statistic for the hybrid Buda \times Ottawa 770B is 0.290 ± 0.312 and for the hybrids Cyprus \times Ottawa 770B and Cyprus \times Buda -0.117 ± 0.288 and $-0.184 \pm$

TABLE VII
MEAN AND VARIATION IN WEIGHT PER THOUSAND KERNELS IN GRAMS OF PARENTS
AND HYBRID LINES

—	No. of lines	Mean weight, gm.	S.E.	C.V.
Buda	25	4.09	.10	2.4
Ottawa 770B	50	5.99	.27	4.5
Cyprus	53	9.22	.35	3.8
33-215 Ott. 770B \times Cyprus	35	7.04	.53	7.5
33-216 Cyprus \times Ott. 770B	34	7.02	.56	8.0
33-217 Buda \times Ott. 770B	58	4.55	.32	7.0
33-73 Buda \times Cyprus	54	6.34	.49	7.8

TABLE VIII
DISTRIBUTION OF HEIGHT OF PLANT IN INCHES OF PARENTS AND F_3 LINES

Class	No. lines	Classes for height in inches																				Mean
		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
Buda	50																					26.84±0.17
Cyprus	50					1	3	12	17	12	1	4										19.10±.19
Ottawa 770B	50									1	2	2	9	9	14	10	2	1				24.58±.20
33-215																						
Ott. 770B × Cyprus	35	1	1	3	6	4	8	3	3	1	2	2	1									17.06±.45
33-216																						
Cyprus × Ott. 770B	34			1	3	1	5	4	9	5	2	1	2	1								18.73±.41
33-217																						
Ott. 770B × Buda	58									2	3	5	14	9	8	5	2	4	4	2		24.17±.38
33-218																						
Buda × Ott. 770B	35											2	1	1	5	2	5	7	7	2		27.51±.28

0.316 respectively. While the small number of lines in each class makes it difficult to draw any definite conclusions on this character, these data do not follow those of Myers (18). In a cross of Ottawa 770B with Redwing he has reported evidence of a partial dominance of large seed size.

Height of Plant

The Cyprus variety is a short-strawed type. In studying the cross with Ottawa 770B, a medium tall variety, a comparison of height of F_2 plants with the mean height of their F_3 progenies showed a correlation of 0.354, with the 1% level at 0.325. This would seem to indicate that height of plant can be explained on a genetical basis. The distribution of the F_3 lines with their parents, grown in the season of 1935, is shown in Table VIII. The variability of the hybrids is greater than that of the parents, showing that segregation may have taken place. The fact that many plants are in lower classes than the parents and also that the mean height is less than that of the shorter parent would suggest a dominance of short-strawed plants. This is especially true when we consider that many of the segregates are in the heterozygous condition and might still be expected to show hybrid vigor.

Association of Characters

Relation of Iodine Number to Oil Content

The tendency of varieties producing large quantities of oil to produce oil of poor quality has already been indicated. Studies of partial correlation indicate that these characters are not correlated. The oil content of the parents did not differ to a very great extent. The correlation of oil content and iodine number within the different lines of the Cyprus parent was -0.388, of the Ottawa 770B parent 0.334, and of the Buda parent 0.314; with the 5% level at 0.444, 0.396, and 0.388 respectively. This agrees with Ermakov (6) who found no association of these characters among the different lines within a variety.

The correlation between oil content and iodine number in the progeny of Cyprus \times Ottawa 770B was 0.163 and 0.141 where a correlation of 0.325 or higher is required to show significance. In this instance iodine number may have been inherited independently of the percentage of oil in the seed. Cole *et al.* (3) found that selection for quality in the oil of the soybean did not tend to depress the quantity present.

Relation of Color of Seed to Quantity and Quality of Oil

From the F_3 generation the nature of the selection in the F_2 was determined, *i.e.*, whether they were true breeding brown or yellow or were heterozygous for color of seed. Iodine numbers of these lines are summarized in Table IX.

The significance of the difference of the yellow compared with the heterozygous and the brown classes was calculated for each group of hybrids. In comparison with the P value of 0.01, it will be seen that these differences in the means of these groups are significant, especially in the cross Ottawa 770B \times Buda. There was a tendency for a higher mean iodine number to be associated in each cross with yellow seed.

TABLE IX

IODINE NUMBER OF YELLOW-SEEDED LINES COMPARED WITH BROWN AND HETEROZYGOUS LINES
IN THE F_3 GENERATION OF OTTAWA 770B \times CYPRUS AND OTTAWA 770B \times BUDA

Seed color	No. of lines	Mean iodine number	S.E.	<i>t.</i> value	P = 0.01
<i>33-215 Ottawa 770B \times Cyprus</i>					
Yellow	10	183.7	5.21		
Heterozygous	15	177.8	5.62	2.64	2.807
Brown	10	178.2	3.15	2.86	2.878
<i>33-216 Cyprus \times Ottawa 770B</i>					
Yellow	11	182.3	4.06		
Heterozygous	16	174.9	4.53	4.28	2.787
Brown	7	177.9	7.52	1.63	2.921
<i>33-217 Ottawa 770B \times Buda</i>					
Yellow	27	194.2	2.77		
Heterozygous	21	187.6	2.79	8.16	2.575
Brown	10	188.4	2.35	5.87	2.575

Relation of Color of Oil to Quantity and Quality of Oil

From a practical standpoint, oil of high iodine value, low in pigment content, is desirable. However, in the hybrid progenies studied, neither the quality of the oil nor the oil content of the seed appears to be associated in any way with pigment content. The correlation between carotene pigment, measured in parts per million, and oil content was -0.204 while with iodine number it was 0.035 . Both correlation coefficients are below the 5% level for significance.

Relation of Size of Seed to Quantity and Quality of Oil

Several investigators (1, 4, 13) have pointed out an association between seed size and percentage of oil, emphasizing the importance of seed size as an aid in selection for high oil content. It has already been shown in a survey of varieties that large-seeded varieties tend to be higher in oil content. Ermakov (6) has been unable to show a similar association when large- and small-seeded types within the same variety are analyzed. In this study the oil content and iodine number have been considered from lines of the parent and hybrids grown under the same environmental conditions. As shown in Table X, neither the parents nor the hybrids show any tendency for size of seed to be related to oil content or iodine number. The lack of association between these variables should facilitate the possible combination of these desirable characters.

TABLE X

RELATION OF SIZE OF SEED TO OIL CONTENT AND IODINE NUMBER. OIL CONTENT IN PER CENT (*o*); IODINE NUMBER (*i*); WEIGHT PER THOUSAND KERNELS (*m*)

	r_{mo}	r_{mi}	5% level
Buda	-0.29	-0.35	0.39
Cyprus	-.19	-.27	.44
Ottawa 770B	.15	.19	.39
33-215 Ott. 770B × Cyprus	.16	.04	.35
33-216 Cyprus × Ott. 770B	-.19	.16	.35

Relation of Height of Plant to Quantity and Quality of Oil

In studying the relation of height of plant it was found that oil content was negatively and iodine number positively associated with height. The segregation of factors for height has already been indicated. In the reciprocal crosses of Cyprus and Ottawa 770B, correlations of -0.293 and 0.001 have been obtained between height and iodine number. In the cross Buda × Ottawa 770B the correlation was -0.347 . Since a correlation of 0.349 or higher is required to show significance, no relation between height of plant and iodine number is indicated.

Discussion

The purpose of this project is to study the relation of quality and quantity of oil to other plant characters in flax and to determine their mode of inheritance. A highly significant positive correlation for high quality and quantity of oil would greatly facilitate selection for this combination. The results from the present survey of varieties are in agreement with those of Johnson (14) in indicating that the simple correlation is negative and significant while the partial correlation is not. Added information was obtained from the hybrid progenies of Cyprus × Ottawa 770B where segregation has been shown to have taken place. However, no association of these characters has been indicated by a study of either the parent lines or those of the hybrids.

Iodine number appears to be affected by several factors, with no suggestion of dominance of high or low quality. This does not agree with the data of Arny, as presented by Dillman (5), who suggests low iodine number to be dominant, and in backcrosses with parents of medium or high iodine number, a ratio of approximately 1 : 1 was obtained. The Bison variety, used by Arny as the parent for low iodine number, is considerably higher than Cyprus and may account for this difference.

Previous investigators (5, 14) have considered it important to select strains of flax with medium-large seed in order to obtain a satisfactory yield of oil. Since no significant relations were obtained between either oil content, iodine number or size of seed in the hybrids studied, greater progress in improvement of the flax crop may be expected from analyses of the hybrid selections. The refractometric methods have been found very practical for such determinations.

There are many agronomic advantages in large-seeded varieties aside from the possibilities of increased oil production. The distribution of seed size is of interest here, since it does not appear to follow that described by Myers (18), who also used Ottawa 770B as one of the parental types. Myers' data suggest a partial dominance of larger seed, whereas, in the distribution shown, in only one instance has the large parental size been regained. A dominance of small size would be in accordance with the recent theory of Fisher (9) that the wild species was evolved by the accumulation of dominant genes. Vavilov (23) has suggested that the wild progenitor of our common flax is the species known as *Linum angustifolium* in which the seed averages 1.50 gm. per thousand kernels. Vavilov believes that domestication is the result of the selection of recessive genes.

The few varieties of yellow-seeded flax that have heretofore been grown in Canada have been observed to yield oil of very high iodine value. Data from the segregation of these two characters indicate a very definite association of high iodine number with yellow-seeded segregates. Recently, Dillman (5) has published data of Arny which show an apparent linkage of yellow seed with high or medium iodine number. In a study of flower and seed type it has been shown both here and in other investigations (18, 21) that the inheritance of the white petal, involute flower type with yellow anthers and greenish yellow seed can be explained by the action of a basal factor or group of factors very closely linked. The morphological effects of this factor or group of factors might now be extended to include the physiological effect of quality in the composition of the oil.

Yellow-seeded varieties appear generally to be considerably lower in oil pigmentation than brown-seeded varieties. This may be of particular interest to the manufacturer of lightly colored paints and enamels. Morrell and Wood (17) point out that it is most difficult to obtain a bright pale oil that will give satisfaction in drying properties. Sometimes one of these properties is sacrificed at the expense of the other. The color specification for a refined linseed oil is not darker than 0.1 gm. potassium chromate dissolved in 100 gm. of sulphuric acid of a specific gravity of 1.84. In studying pigment content in a preliminary manner with the cross of Ottawa 770B \times Buda there are indications that "carotene" pigment content may be dependent on several factors for its expression. The color of the oil was not correlated with either oil content or iodine number. The fact that yellow-seeded segregates with very high pigment content were obtained would indicate that oil color may also be independent of the color of the seed.

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CHEMICAL WEED KILLERS

II. FACTORS AFFECTING ESTIMATION OF TOXICITY OF LEAF SPRAYS¹

By W. H. Cook²

Abstract

Chemicals applied to annual weeds frequently gave dosage-mortality curves that were not of the usual sigmoid shape. These departures appear to be attributable to the method of application, as the spray is only partly retained by the leaves and stems. The proportion of the applied quantity of poisonous constituent retained by the plant decreases as the volume of spray is increased, and increases as the concentration of the spray solution is increased. Leaf sprays will therefore be most effective if the minimum volume of solution required for coverage is used, and the effective dosage of the chemical obtained by adjusting the concentration.

A few indices sometimes used for estimating the efficacy of herbicides were compared with the mortality criterion. The number of leaves left, and the height of the living plants after treatment are of little or no value for estimating the effect of the chemical. The weight per unit area of the living plants remaining after treatment may be of some value.

Introduction

A review of the literature (4) shows that there is a great divergence between the dosages of a given chemical reported by different investigators to kill a certain species. In most cases this divergence is so great that it has been impossible to reach a definite conclusion concerning the relative toxicity of different chemicals. This variability appears to be attributable to three main causes:

1. The variation in the susceptibility of plants of the same species. It appears that most investigators studying chemical herbicides have failed to appreciate the magnitude of the variability that is encountered when apparently similar organisms are used. Added to this variation between individuals, is the variability contributed by the different soil and climatic conditions under which different experiments are carried out.

2. Drainage to the soil of a variable proportion of the applied spray. Considerable variability can be expected from this source since the relative proportion and efficacy of the poison applied to the aerial parts of the plant and to the soil are unknown.

3. The various criteria of effectiveness used by different investigators. Some experimenters report the percentage mortality resulting from a given treatment, some report the weight, height or other measure of growth, others the growth rate, yield of economic crop following treatment, etc. Although

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the mortality criterion is the one most commonly used in precise studies of toxicity, and is the one that has been studied most from the standpoint of suitable methods for estimating the effective dosage and its variability, it cannot always be employed in herbicide studies under field conditions. It is possible, however, to gain some idea of the relation between mortality and the other criteria, so that the significance of the results can be estimated.

It is evident that little can be done toward reducing the variability arising from the different susceptibility of individual plants of one species, but the error arising from this source can be decreased by adequate replication. The form of the dosage-mortality curve is determined by the variation in susceptibility, and if the usual sigmoid curve is obtained, methods such as those of Trevan (5) and Bliss (1, 2) can be applied to estimate the effective dosage, and to evaluate the extent to which these estimates may be in error. If the dosage-mortality curve is not sigmoid, consideration must be given to the possibility of other systematic errors affecting the results. A few of these are discussed in this paper.

Most of the experimental data presented were obtained in connection with an investigation into the relative toxicity of different chemicals to annual weeds. As the materials and methods employed have been reported in an earlier paper (3) they need not be described here. It is important to note, however, that the chemicals were usually applied as a 10% solution, and the dosage was varied by adjusting the volume of solution sprayed on the plants.

The author regrets that he had no opportunity to determine whether the hypotheses presented are applicable under field conditions. Nevertheless, they are presented here in the hope that they may be useful.

The Dosage-Mortality Curve

In most cases where a toxic substance has been administered directly into the organisms, or where the latter can be subjected to a known concentration of the poison, as in fumigation, a sigmoid dosage-mortality curve has been obtained. This represents the integrated frequency curve descriptive of the variable susceptibility of the individuals tested. The exact position, shape and slope of this curve usually differs somewhat for each poison and species under investigation, but in general the greatest slope is in the region of 50% mortality, and comparative tests of different substances can generally be made most accurately in this region. During the course of the investigation already mentioned (3) an attempt was made to determine the shape of the dosage-mortality curve obtained with certain species and chemicals. It was found that these curves often departed considerably from the usual sigmoid form, and in consequence the certainly lethal dose, rather than the median lethal dose, was used as the measure of toxicity and, with two exceptions, no attempt was made to determine the dosage-mortality curves for all chemicals and species. Owing to the number of dosages employed in certain instances, however, sufficient data were collected to permit the nature of the curves to be approximately determined. These curves are shown in Fig. 1.

Chart A in the upper left-hand corner shows the curves obtained from the application of formic acid and zinc sulphate to stinkweed, *Thlaspi arvense* L., and formamide and sodium acetate to wild mustard, *Brassica arvensis* (L.) Ktze. These two weeds have about the same resistance to toxic sprays, and were the most susceptible annuals tested (3). All of these curves are approximately sigmoid with the exception of that for the least toxic substance, sodium acetate.

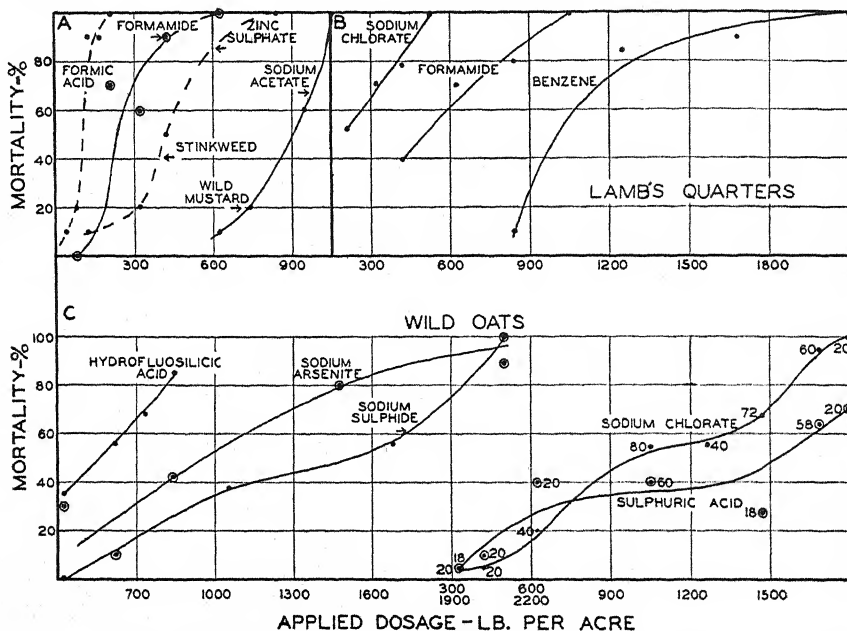


FIG. 1. Typical dosage-mortality curves for chemicals applied as a spray of constant concentration.

Chart B (Fig. 1) shows the effect of three chemicals on lamb's quarters, *Chenopodium album*, L., which is approximately twice as resistant to chemicals as stinkweed and wild mustard. The two more toxic materials show a practically linear relation between 50 and 100% mortality. On the other hand the curve for benzene rises steeply at first, but gradually decreases in slope as it approaches 100% mortality.

Chart C (Fig. 1) represents curves obtained with wild oats, *A. fatua* L., which is approximately seven times as resistant as stinkweed and wild mustard, judging from the certainly lethal dose applied as a spray. On the left-hand side the approximate nature of the curves for hydrofluosilicic acid, sodium arsenite and sodium sulphide has been drawn from the few data available. Of these, hydrofluosilicic acid and sodium arsenite show an approximately linear relation between dosage and mortality. Sodium sulphide, on the other hand, shows a slight tendency to flatten out in the region of 50% mortality. The two curves in the lower right-hand corner represent the data obtained from spraying with sodium chlorate and sulphuric acid. Many

more data were available for plotting these two curves, and at least their shape can be fixed with some degree of certainty. The number appearing beside each point represents the number of individual plants used for its determination. It is obvious that they are not sigmoid, both tending to flatten out in the region of 50% mortality.

The main point illustrated by Fig. 1 is that a sigmoid relation between dosage and mortality was obtained when the more toxic chemicals were applied to the more susceptible species of plants. As the resistance of the species under test increases, or the toxicity of the chemical decreases, this relation disappears and the curves assume various shapes, but finally tend to flatten out at a certain mortality, and rise again at high dosages. The shape of these curves could be attributed to a decided asymmetry in the distribution of the susceptibility of the individual plants, but it seems more likely that the first portion of the curve results from the action of the spray on the leaves, while the portion of the curve beyond the flat part represents the additional toxic effect of the chemical in the soil. This leads to a consideration of the second source of variability, namely, the method of application.

Relative Amounts and Efficacies of Spray Retained by Foliage and Entering Soil

The application of a toxic solution to growing plants as a spray results in a certain proportion being retained on the plant, while the rest drains to the soil. The proportion of the spray remaining on the plant may be termed the "retained" dosage and will be affected by many factors such as the size of the plant, density of growth, type of leaf and stem, the retentive properties of the plant surfaces for the toxic solutions, etc., but for the most part it will be determined by the volume of spray required to carry the effective dosage of the poison. The remainder of the applied dosage will drain to the soil where it may be detoxicated, or exert an additional toxic effect. This brings up the question of the relative efficacy of chemicals applied directly to the foliage, or indirectly through the soil. Doubtless this varies greatly with different chemicals and soils. It seems likely, however, that most of the leaf sprays, or contact poisons, act mainly on the foliage when applied in the dosages ordinarily used, but it must be recognized that most of these substances could potentially exert a toxic action through the soil if applied in sufficient quantity. Many such substances can be detoxicated in the soil by neutralization, adsorption, conversion to insoluble compounds, etc., while dilution in the soil solution affects all poisons, and could easily reduce the concentration of small dosages below the minimum required to be effective. These considerations indicate that the dosage required to produce a certain mortality through the soil would generally be higher than that required on the foliage. Under these conditions, the part of the solution draining to the soil will be lost if small volumes are applied, but may produce an additional toxic effect if large quantities are used. Such effects could easily modify the shape of the dosage-mortality curve. Certain chemicals may act through the soil only, but the consideration of these is beyond the scope of this paper.

It is evident that some method of estimating the "retained" dosage from the "applied" dosage of leaf sprays is required. The maximum amount of spray which plants of a given species, of size A (in terms of surface area of plant per unit area of land), will retain is unknown, and there are no data available from which it could be estimated, but it can be designated as V_m gallons per acre. Now let it be assumed that:—

(1) The density of plant growth is such that the entire surface presented to the spray is plant leaves or stems, so that none of the spray falls directly on the soil, but reaches it only by drainage from the plants.

(2) All portions of the surface area of the plant are equally accessible to the spray.

(3) Any excess of liquid on any portion of the plant surface will drain off directly and not wet an otherwise dry area.

The last two assumptions are not strictly justifiable but they are sufficiently correct for purposes of estimation, and since they act in opposite directions, the resulting inaccuracies will tend to cancel each other.

Since an increment of spray may fall with equal probability on any portion of the entire surface area of the plant, it follows that the amount retained will be that held by a constant proportion of the dry area remaining at the time of application, while the dry area will decrease continuously as the applied volume increases. The amount retained out of each succeeding applied increment will therefore diminish continuously. The relation between retained and applied volumes can therefore be represented by the general equation for continuous depreciation of the type:

$$dR = e^{-V} dV,$$

where dR is the quantity retained when an increment dV of solution is sprayed on after V gallons per acre have been applied. In this equation all constants expressing the retentive abilities of the system under consideration are unknown and have been taken as 1, so that only the shape of the retention curve, and not its position and slope, are described by the equation.

Integrating the above equation gives

$$R = 1 - e^{-V}$$

where R is the amount retained after V gallons have been applied, while the proportion of the total applied spray retained is

$$\frac{R}{V} = \frac{1 - e^{-V}}{V}.$$

In all cases the proportion of the spray draining to the soil can be obtained by difference.

The retention curves can be deduced from the above equations by plotting the proportion retained, for different values of V , against V_m as defined earlier. These curves appear in B, Fig. 2, and it can be seen from the retention curve (R) that when a quantity of spray equal to V_m , the maximum amount

that can be retained, has been applied, only about 63% of it remains on the plant. When a quantity equal to $3V_m$ has been applied the portion retained is about $0.95V_m$, or about one-third of the spray employed. If still larger volumes are applied most of the spray in excess of $3V_m$ drains to the soil.

Since the proportion of the spray draining to the soil will be ineffective as a leaf spray, the retained dosage rather than the applied dosage should be employed for constructing dosage-mortality curves for substances acting only on the leaves. In A, Fig. 2, two symmetrical distributions of the susceptibility

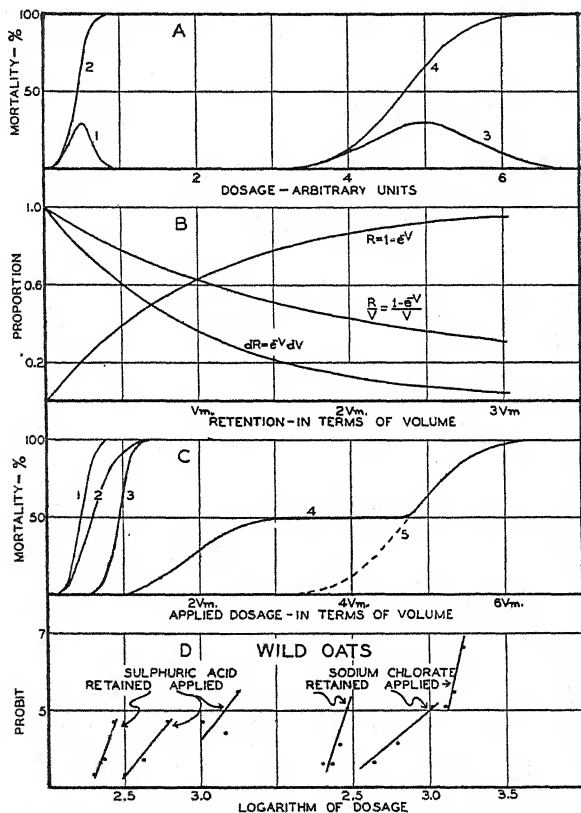


FIG. 2. Effect of partial retention on the form of the dosage-mortality curve.

of individual organisms to a hypothetical poison have been constructed (Curves 1 and 3), together with the integrated frequency, or sigmoid dosage-mortality curves obtained from these distributions (Curves 2 and 4). In both cases it is assumed that the entire dosage was retained by the organism, or administered into it, so that the distribution is not affected by the difference between the "applied" and "retained" quantities. The left-hand distribution and mortality curves apply to a relatively toxic material having a median lethal dose in the region of 0.5 dosage units. The right-hand curves apply to a less toxic substance having a median lethal dose in the region of five dosage

units. The variation in the susceptibility has been assumed to be proportional to the decreased toxicity, which results in the distribution shown in the graph. Although these examples may be somewhat exaggerated, Curves 2 and 4 are analogous to what might be expected if a typical leaf spray were applied to the aerial parts of the plant, and to the soil, respectively.

In C, (Fig. 2) the mortality resulting from the application of hypothetical substances is plotted against the applied dosage in terms of V_m . Curve 1 is a reproduction of Curve 2 in A, and would be the type obtained if all of the toxic spray was retained. By computing the proportion of the spray retained by the plant from the curves in B, and assuming that only this portion of the spray was effective, the dosage-mortality curve is changed in slope and position to that represented by Curve 2. This changed position suggests a new distribution of the susceptibilities, but it is, in fact, due entirely to the difference between the applied and retained dosages. Since the discrepancy between the retained and applied dosages increases as the volume of spray is increased, the divergence between the "observed" and "real" dosage-mortality curves will increase as the toxicity per unit volume of the solution decreases. Since the plant can retain only the volume V_m gallons of spray per acre, it is obvious that if this volume does not contain sufficient chemical to kill even the most susceptible plants, no mortality will result until the material is added in sufficient quantity to act through the soil. If, however, the quantity V_m carries sufficient poison to kill a certain percentage of the more susceptible individuals, this percentage mortality will be approached asymptotically, and will be reached, for all practical purposes, when about $3V_m$ gallons have been applied, since this corresponds to a retained quantity of approximately $0.95 V_m$. This behavior is illustrated in Curves 3 and 4 in C, Fig. 2. Curve 3 depicts the same distribution as Curve 1, but has been moved to the right, so that the median lethal dose corresponds to the amount contained in V_m gallons of spray, and is again obtained by assuming that all of the spray is retained by the plant. Since only part of the applied dosage is retained, the mortality, plotted against the applied dosage, will be represented by Curve 4, and will reach a constant mortality in the region of $3V_m$ applied dosage. Obviously, different chemicals, or solutions varying in toxicity per unit volume, can produce dosage-mortality curves which reach constant mortality values anywhere between 0 and 100% mortality.

Although certain toxic sprays may produce only a partial mortality from their action on the leaves and stems, owing to the limited retaining power of these parts, it is also possible that some may show an increased mortality at extremely high dosages from their action through the soil. In order to illustrate the effect of the combined action on the shape of the dosage-mortality curve, Curve 4 in A has been assumed to represent the dosage-mortality relation when the chemical acts through the soil only, and has been reproduced as Curve 5 in C. The flat portion of Curve 4 in C, representing the partial mortality at the maximum retained dosage, intersects this curve, and if the substance in question can act through the soil, the complete dosage-mortality

curve will, as a limiting condition, follow the solid line representing the combination of Curves 4 and 5. Any interaction of the two toxic effects will alter the shape of this combined curve near the point of intersection, but no data are available on this point. The point of intersection of the two curves will naturally depend on the mortality produced from the action of the chemical on the leaves, the effective dosage in the soil, and any tendency for the action at the two loci to be additive. The general similarity in shape between the solid line formed by combining Curves 4 and 5 in C, and the curves obtained experimentally with sodium chlorate and sulphuric acid applied to wild oats (Fig. 1), supports the several hypotheses here deduced from theoretical considerations.

A further study of the dosage-mortality curves was made by plotting the probit, or probability unit, against the logarithm of the applied dosage. These conversions have been used by Bliss (1) to convert sigmoid dosage-mortality curves to straight lines suitable for precise statistical treatment. The probit is essentially the number of standard deviations describing the distribution of the variable susceptibilities, chosen in such a manner as to avoid negative values. The logarithm of the dosage, rather than the dosage itself, has been used, since the majority of the experimental data so treated have been found to yield a straight line, and the inference is that the susceptibilities are distributed as a logarithmic, rather than as an arithmetical, function of the dosage. When this double conversion was applied to the sigmoid curves in Fig. 1, the resulting curves were slightly concave upwards, regardless of whether "applied" or "retained" dosages were considered, but as these curves were based on rather few data, the applicability of the double conversion to herbicide tests must await the results of further experiments. When the double conversion was made with the dosage-mortality curves based on applied dosages of sodium chlorate and sulphuric acid (Fig. 1), the results obtained with both chemicals could be fitted graphically by two straight lines, which appear in D, Fig. 2, and support the contention that the original curves are the result of two separate effects, *i.e.*, one on the aerial parts of the plant, and the other through the soil.

The straight line representing the first part of the dosage-mortality curve, and attributed to the action of the chemical on the leaves, was converted to the retained dosage, using the curves in B, Fig. 2, and assuming V_m to be 300 gallons per acre. In order to arrive at this value of V_m , the dosage at the beginning of the flat part of the curves in Fig. 1 was taken as $3V_m$. This gave V_m as 220 and 350 gal. per acre for sulphuric acid and sodium chlorate respectively. Some preliminary direct measurements made on similar plants after spraying 1000 gal. per acre indicated that the retained quantity lay between 120 and 320 gal. per acre. The curves plotted from the "retained" quantities are altered in position, and have a considerably greater slope. Extrapolation of these lines indicates that the C.L.D. to wild oats of both sulphuric acid and sodium chlorate when applied to the leaves only is about 850 lb. per acre. Little significance can be attached to this value until V_m , and

other properties of the chemicals under investigation, are known more accurately. For example, sodium chlorate is generally believed to act almost entirely through the soil, and it is also known to act slowly compared with sulphuric acid. Both of these factors may affect the shape of the dosage-mortality curve, and may account for the fact that 500 lb. per acre of sodium chlorate applied to weeds was found to kill wheat planted in the soil shortly afterwards (3), whereas the curve in Fig. I shows only a 55% mortality of wild oats following applications of 1000 lb. per acre. On the other hand, this difference could also be explained by a difference in the susceptibility of the two species of plants.

The foregoing considerations show that the loss of toxic solution from the foliage must affect the efficacy of herbicides. If the chemical acts through the soil only, the concentration of the solution is of secondary importance, but if it is used as a leaf spray the concentration must be such that a lethal dosage of the substance will be retained by the plant. Furthermore, since the proportion of spray reaching the soil increases with the volume of spray used, this loss will be reduced by using as concentrated a solution as possible. On the other hand, a sufficient volume of spray must be applied to give at least the minimum coverage required to produce complete mortality. The best concentration to use in practice will therefore be one containing a certainly lethal quantity of the chemical dissolved in a volume of solution somewhat in excess of that required to give the minimum effective coverage. It is therefore necessary to determine experimentally the volume of solution required for effective coverage and the maximum retained volume, for a given size of plant of the species in question, before the C.L.D. can be determined by varying the concentration. In this connection, the size of the plant is extremely important, and in the past has not received the consideration it merits. Its estimation by cutting and weighing the plants from small areas selected at random would permit some standardization of the lethal dosage and volume required, as functions of plant size rather than per unit area. Since the chemical must affect a certain proportion of the cells, or constituents of the plant, it seems reasonable to believe that the lethal dosage will change in proportion to the weight of the plant. The capacity of the plant for retaining the toxic spray will vary as the area of the plant surfaces changes, but since the major part of the growth of most weeds is in two dimensions, it seems likely that the area and weight will be closely related. Hence when the lethal volume and concentration of spray for a given size of plant are known, the dosage required for other sizes of plants will be obtained sufficiently accurately if the volume of solution only is changed proportionately.

Although concentrated solutions might result in retention of a greater proportion of the toxic constituent, it is possible that the absorption of the poison by the leaves may be the factor limiting the efficacy of certain substances. Where this is the case, concentrated solutions might be of little value, but further investigation is required before a definite statement can be made.

Criteria of Toxicity

The use of different criteria by different investigators (4) renders many of the published results non-comparable since the relation between the quantities measured and the mortality are unknown. For instance, a reduction of 50% in the weight of plants per unit area might not result in the death of any of the plants.

In the first paper of this series (3), reporting work in which the certainly lethal dose was used as an index of the relative toxicity of different substances, the results of a considerable number of dosages leading only to partial mortality were omitted. In these tests the green weight of the plants, their height and the number of leaves per plant, were observed and expressed as a percentage of the same quantities determined on the untreated control plants grown at the same time under similar conditions. The relation between these quantities and the observed mortality for each of the four kinds of plants studied, are shown in a series of scatter diagrams in Fig. 3. Each point

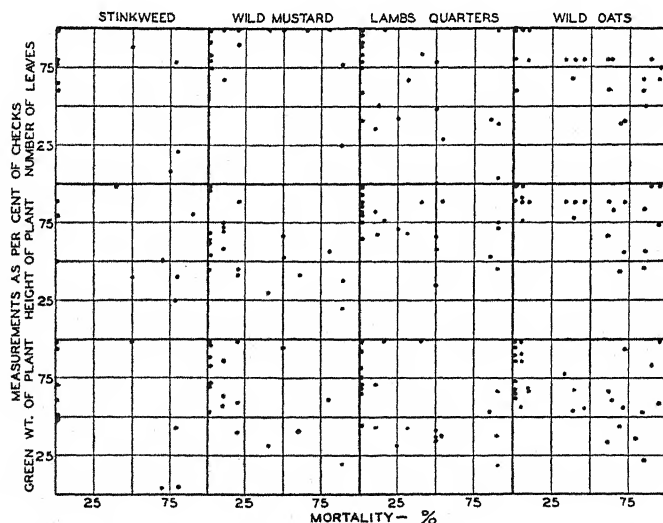


FIG. 3. Relation between mortality and other criteria of toxicity.

represents the results for a given dosage of a given chemical. All the chemicals are grouped together in the appropriate diagram; no attempt has been made to distinguish between them since any reliable measurement of the efficacy of the poison must apply to all substances if it is to be generally useful. Two or three of the points on some of the diagrams represent the effect of one substance applied at different dosages.

Accepting mortality as the standard criterion of toxicity, it is evident that the suitability of any new criterion will depend on the degree to which it is correlated with mortality, and also on the constancy of this relationship for different species of plants. Examination of Fig. 3 shows that while there is some agreement between the various criteria and mortality for some of the

species, the variability is so great that the degree of correlation would never be high. With respect to the constancy of the relations between different species, it appears that each of the weeds differs somewhat, but from this standpoint the value of the criteria perhaps increases in the order: number of leaves, height of plant, weight of plant. When the high variability between the various criteria and mortality, and between different species, are both considered, it appears that the weight index is the only one that might be useful as a measure of toxicity.

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THE PROTECTIVE LAYERS OF THE APPLE¹

BY HUGH P. BELL²

Abstract

The development of the four protective layers of the apple, namely the hairs, cuticle, epidermis and hypodermis, is described in detail, from the time the flower emerges from the winter bud early in May, until harvest time early in October. The gradual loss of living and active cell contents is recorded for each layer. Measurements of cuticle thickness for the period of the study are listed and an approximate date is given for each stage of development. The descriptions of tissue morphology are supplemented by ten illustrations.

Introduction

The structure and development of the protective layers of the apple are fairly well known, but there is a great variation from locality to locality in the time of the year when each stage in this development is reached. The condition of these protective layers at any one time has an important bearing on the reaction of the young fruit to sprays and weather conditions to which it may be exposed at that season, hence, for each locality, it is desirable to know the approximate date when each protective tissue is developed. The study outlined below was undertaken at the request of the Pathologist-in-Charge at the Laboratory of Plant Pathology, Kentville, N.S., to determine the rate and time of development in Nova Scotia.

The apple studied was the McIntosh Red. The material was collected during the summer of 1934, by the staff of the Laboratory of Plant Pathology, Kentville, N.S. It was killed in chrom-acetic, and imbedded in paraffin as described by Bell and Facey (1). The stains used were acid fuchsin for cell wall, soudan IV for cuticle, and iron alum haematoxylin for cell contents. During the summer of 1934, the trees from which the material was collected were in full bloom on June 2, and the fruit was ripe and harvested by the end of September or early in October.

The development of the protective layers could be traced from the first appearance of the flower primordia, but there is no practical interest in these layers while they are still completely enclosed in and covered by the scales of the winter bud. Interest in these layers starts when the surface of the young apple is first exposed to weather conditions and to early sprays. Hence the earliest condition described in this study is that prevailing just at the time the young flowers start to emerge from the winter buds. In the spring of 1934, when the collections for this study were made, this stage was reached during the second week of May, that is about three weeks before full bloom.

The outer protective region of the apple includes four layers of tissue. These are: the coating of epidermal hairs, the cuticle, the epidermis and the hypodermis. The development of each layer will be described by itself and

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then, to give an idea of the structure of the protective region as a unit at significant periods of the growing season, the information will be presented in tabular form. The findings recorded below agree in general with those of Tetley (4, 5), who reported on the development of the protective layers for a number of varieties of apple commonly grown in England. A few minor disagreements were noticed regarding certain details. These will be mentioned as each structure is discussed.

In layers that are cellular in structure, the type of reaction to changes in growth and environment depends largely on the condition of the protoplasmic cell contents. For instance, active cells can repair minor ruptures or injuries by the growth of new cells, but these active cells are very sensitive to an unfavorable environment of either weather or sprays. Conversely thick-walled semi-living cells cannot as a rule repair a rupture in their layer, but they are highly resistant and are not easily injured by an unfavorable environment. Hence while these morphological changes are taking place, it is important to know the extent to which the cells retain their vital properties, and an attempt has been made to record this information for each protective layer and for each stage of development. The protoplasmic contents were used to indicate the condition of the vital activity of the cell. Where cell division could be demonstrated, the cells were regarded as active. Where the nucleus and other protoplasmic contents had completely disintegrated, the cells were judged to be dead or at the most semi-living. All stages between these two extremes were observed.

The Development of Each of the Four Protective Layers

The Coating of Hairs

At the time the young flowers start to emerge from the winter buds, the ovary is covered with a dense mat of tangled hairs. Each hair is a modified epidermal cell and is living and active. The proximal portion of a typical hair as it exists at this time is shown in Fig. 1. The surface of the hairs is such that it is not easily wetted, and in addition this layer encloses myriads of minute and more or less enclosed air spaces. Consequently this mat of hairs is a very efficient protection to the young ovary. As the ovary enlarges, the epidermal cells between the hair bases multiply by cell division. As a result the hairs become more widely separated. Another important change takes place during the three weeks immediately preceding full bloom. The layer of cuticle that is being laid down over the outer surface of all the epidermal cells is comparatively much thicker just where the hair base joins the outer tangential wall of adjacent epidermal cells, and this comparatively thick layer of cuticle extends into the hair base and down the inner surface of the radial walls till it reaches the inner tangential wall at the base of the cell. This is illustrated in Fig. 4. Thus, at the time of full bloom, although the ovary is still densely tomentose, the cuticle has already started to replace the hairs as the outside protective layer. Also as the hairs are now practically cut off from other living cells, they are not so active or efficient as they were

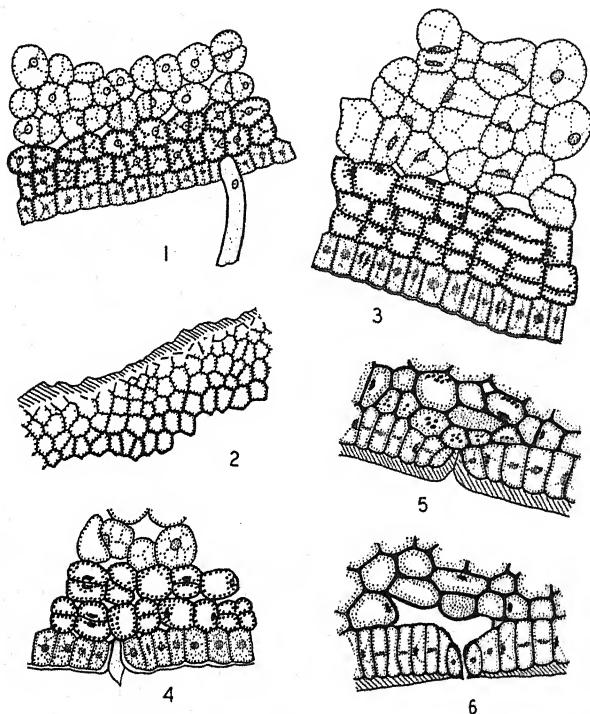
at first. This is indicated by the fact that they are gradually becoming more brittle and more easily wetted. The deposition of cuticle continues and by the middle of June the space formerly occupied by the hair base is completely closed. This closing of the basal cell continues, until finally the location of the hair base cannot be identified with certainty. Apparently this formation of cuticle does not of itself cause a separation of the hair from the surface of the ovary, for the proximal portion of the hair may still be found attached even after the cuticle is quite thick, but by the middle of June the slightest touch will cause the hair to break across just at the outer surface of the cuticle. Tetley reports (4, pp. 163, 165, 286) that in the varieties she studied, a rupture in the protective layer frequently occurred at the base of the hair. This was not observed in the McIntosh material examined during this study but, as stated above, the cuticle always filled in the hair base so that the sub-epidermal layers were never exposed and the hair base was finally completely filled in and covered over by a smooth and continuous layer. During the latter part of June the hairs gradually disappear from all exposed parts of the apple, but they may still be found, well on into the summer, in the depressions at the calyx and stalk ends. By that time they are so widely separated and so insecurely attached, that they have ceased to afford any protection.

The Cuticle

At the time the young flowers start to emerge from the winter buds, the cuticle cannot be identified with certainty if one relies on a cross section only (Fig. 1), but it is present at that time, though it is not of uniform thickness. To see it, it is necessary to make a tangential section, then by using soudan IV, a distinct layer of cuticle will be seen (Fig. 2). This layer gradually thickens until by the time of full blossom it is from 1 to 1.7μ thick, and by using soudan IV it can be demonstrated in cross section (Fig. 3). However it is still not a uniform layer for it varies in thickness from spot to spot. This unevenness of the first cuticular deposit does not agree with the findings reported by Lee and Priestley (3, p. 527). They describe the first deposition of cuticle as, "either spreading evenly or thickening where it lies over the line of the middle lamella lying between two contiguous cells." In the McIntosh apple the first deposition of cuticle could be described as almost "patchy", with the thicker patches bearing no relation to the cell structure of the epidermis, and there was no evidence of thickenings over the ends of the radial walls. Also the cuticle at this stage is thicker at the bases of the hairs into which it extends (Fig. 4).

By the third week of June (Fig. 7) the hair bases are all filled in and the cuticle is continuous except over the stomata (Fig. 6) or young lenticels, where it shades off to nothing. It has an almost smooth outer surface, but its inner surface conforms to the irregularities of the epidermis. During August this inner surface of the cuticle starts to invade the epidermal layer in the form of V-shaped wedges between the epidermal cells; this continues till finally, at the time of harvest, the cuticle reaches the hypodermal layer, and may even extend under parts of the epidermis. Its final thickness on the ripe apple is

about 23μ , but owing to the irregularity of its inner surface it is impossible to express this cuticle thickness satisfactorily by one measurement only, for in addition to the inward extensions of the cuticle, the outer tangential walls of some epidermal cells are more convex and protruding than others. Above these protruding cells the cuticle may be very thin. This is not so characteristic of the very early stages, but from about June 8 on, three measurements



FIGS. 1-6. FIG. 1. May 14. The proximal portion of an epidermal hair is included. FIG. 2. May 18. A tangential section. FIG. 3. June 1. In this drawing the unevenness in the thickness of the cuticle is not indicated. FIG. 4. June 1. A hair base. FIG. 5. June 15. A hair base filled by cuticle. FIG. 6. June 15. A stoma. All $\times 290$. In Figs. 2, 5 and 6, the cuticle is singly cross hatched. With the exception of Fig. 2, all are radial sections.

are necessary for a complete picture. These are: (i) the maximum thickness at points exactly opposite the ends of the radial walls between the epidermal cells; (ii) the minimum thickness at points opposite the centres of those epidermal cells which have protruding, convex, outer tangential walls; and (iii) the thickness opposite the centres of what appeared to be normal cells. This third figure is probably the nearest to what could be termed the "thickness of the cuticle". These measurements are given in Table I. Each figure is the average of a number of readings, but all the readings for any one measurement were taken from one specimen only. The information is given in this way to indicate the fact that in different specimens of the same date and even in different parts of the same fruit the thickness of the cuticle may vary

TABLE I
THICKNESS OF THE CUTICLE IN MICRONS

	Over the centre of cells with protruding convex outer walls	Over the centre of cells with nearly flat outer walls	Over the inter- section between two epidermal cells
June 8	2.0	3.0	4.5
June 11	2.4	4.6	5.4
June 15	3.5	4.7	5.8
June 18	4.3	5.8	6.4
June 22	4.7	5.5	6.2
June 25	4.6	6.2	6.8
June 25 }	6.2	7.6	9.0
June 29	6.2	8.5	9.7
July 2	6.2	7.7	10.9
July 6	7.0	9.3	11.7
July 9	5.4	8.5	10.1
July 16 }	7.0	10.9	11.7
July 16 }	7.0	10.1	12.4
July 20	6.6	9.3	12.4
July 23	9.3	12.4	13.2
Aug. 3	8.5	11.7	14.0
Aug. 10	10.9	14.0	18.7
Aug. 17 }	12.4	16.3	19.5
Aug. 17 }	12.4	15.6	20.2
Aug. 24	9.3	15.6	20.2
Aug. 31	12.4	15.6	20.2
Sept. 7	11.7	15.6	20.2
Sept. 21	12.4	16.3	21.8
Sept. 29	18.7	23.4	25.7

May 18. Evident in tangential section, but too thin and uneven to measure accurately.

May 28. About 0.7, but uneven in thickness.

June 1. About 1.0, but uneven in thickness.

June 4. From 1.1 to 1.7, but uneven in thickness.

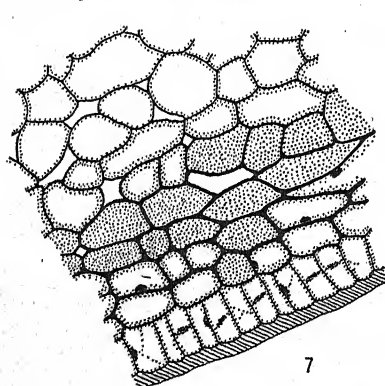
slightly. This is indicated by the figures given for June 25, July 16 and August 17. In general, however, the cuticle increases in thickness at a uniform rate from about the time of full blossom until the maximum thickness is reached about two weeks before harvest.

The Epidermis

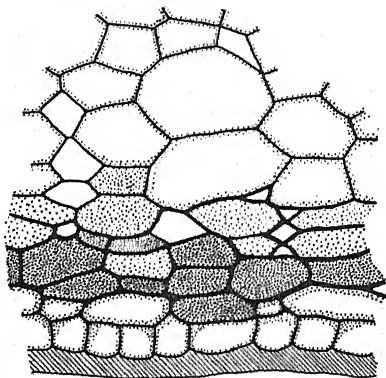
At the time the young flowers start to emerge from the winter buds, the epidermis is a layer of active thin-walled isodiametric cells, measuring from 10 to 11 μ both tangentially and radially. In a section at right angles to the surface, they are rectangular (Fig. 1), and in a tangential section they are round or roughly hexagonal in outline (Fig. 2). During the last three weeks of May, while the young flowers are emerging from the winter buds and the ovaries are starting to increase in diameter, the epidermal cells divide rapidly by radial walls, and mitotic figures may be seen in a number of cells in almost any section. This division by a radial plane is accompanied by a rapid increase in radial measurement, while the tangential measurement may be slightly reduced. The measurements at the time of full bloom are about 18 μ radially, by about 9 μ tangentially. The epidermis is then a closely packed layer of columnar cells and remains as such for two or three weeks.

The greatest radial measurement observed was 23μ in a specimen collected on June 15 (Fig. 5). During these first two or three weeks of June, cell division gradually ceases and the protoplasm becomes less dense, so that by the middle of June (Fig. 7), cell division is no longer observed and although both nucleus and cytoplasm are still distinct, the nucleus is often against the side wall and the cytoplasm is always vacuolated and chiefly peripheral. That is, the cells are still living, but they are becoming less active. There is also a slight increase in the tangential measurement of the cells. At this time (the middle of June) stomata, which appear to be structurally normal, are common throughout the epidermis (Fig. 6). The later condition of these stomata and the development of the lenticels were not followed in this study, but have been described in detail by Clements (2).

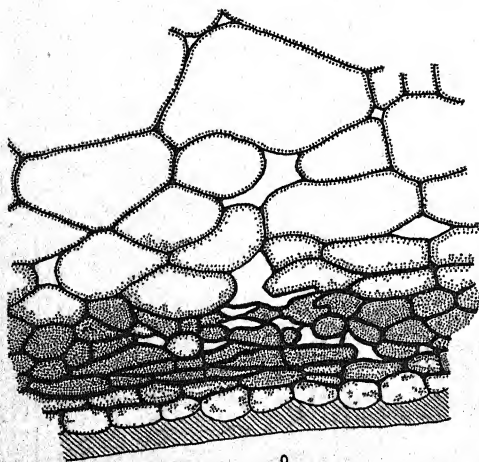
From the middle of June on, the cells of the epidermal layer change rapidly in regard to vital activity, cell contents and cell outline. Cell division could not be detected and all evidence would suggest that it does not again occur in this layer. Accommodation to the rapid increase in circumference appears



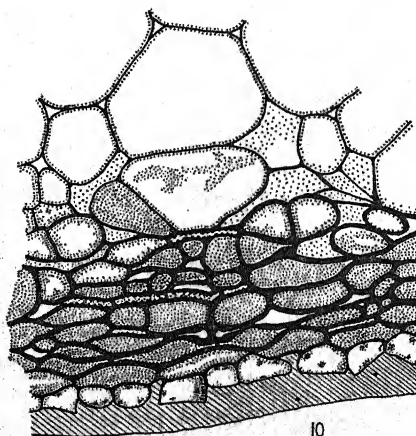
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8



9



10

FIGS. 7-10. FIG. 7. June 27. FIG. 8. July 16. FIG. 9. August 17. FIG. 10. September 29. All $\times 290$. In all figures the cuticle is singly cross hatched.

to be by means of cell enlargement in a tangential direction, so that by the middle of July the epidermal cells have changed from the radially elongated closely packed columnar type seen in Figs. 6 and 7 to the more plate-like tangentially elongated type seen in Fig. 8. At the same time the protoplasmic contents become less dense and extensively vacuolated and it becomes difficult to distinguish the nucleus. At this stage the cells vary greatly in size, but their tangential measurement may reach 32μ and in certain cells the radial measurement may be reduced to 14μ .

During the last part of July and the first part of August, these epidermal cells start to separate. This separation starts on the outer surface and the spaces between the epidermal cells become occupied by V-shaped invasions of cuticle. In places the epidermal cells appear to be grouped in pairs. The protoplasmic cell contents become irregular and disorganized (Fig. 9). During the last part of August and throughout September this process continues. The cells do not continue to elongate tangentially, but they do become more flattened radially. Some even collapse completely. They also become more widely separated, till by the end of September (Fig. 10) the cuticular invasion reaches the hypodermal cells and even extends under the inner wall between the epidermal and hypodermal cells. Also the protoplasmic contents have completely disintegrated and appear as dense or granular patches adhering to the cell wall.

The Hypodermis

At the time the young flowers start to emerge from the winter buds, the hypodermis consists chiefly of two layers of living and active cells (Fig. 1). The outer layer is continuous, but here and there the inner layer may not be differentiated. On the other hand in a few places the hypodermis may be three cells thick. These cells are similar in shape to the adjacent parenchyma cells of the cortex. The only difference is that the hypodermal cells have a thicker wall and their contents are more densely granular. In origin these hypodermal cells are primary tissue, and were originally part of the cortical parenchyma.

During the three weeks immediately preceding full bloom, the hypodermal cells multiply by an indefinite number of radial divisions. This enables the layer to accommodate itself to the increase in circumference without change in cell shape. At the same time each hypodermal cell divides once by a tangential wall. As a result of this tangential division, the average thickness of the hypodermis is increased to four layers, and this is the condition prevailing at the time of full blossom (Fig. 3). The origin of these layers is quite evident, for the daughter cells directly subtend each other in a radial direction, and are so definitely paired that the four rows have the appearance of two double rows. But it should be noted that the original slight variation in thickness is now more pronounced. For where there was originally only one layer there are now two (one double row), and where there were originally three layers there are now six (three double rows). Intercellular air spaces may occur at the corners of the cells (Fig. 4). These air spaces never become

filled with wall material as in typical collenchyma, but later disappear as the result of crushing. The cell walls have thickened slightly and in a section the layer is made conspicuous by the cell contents which are denser and more granular than in either the epidermal or cortical cells. The nucleus and cytoplasm are easily identified and appear to be quite normal. The cytoplasm is vacuolated and largely peripheral. The size of the hypodermal cells at this stage is from 12 to 18 μ tangentially and from 9 to 12 μ radially.

Very shortly after full bloom, the development of the mature features of the hypodermis starts. This development involves the following morphological changes. The cells become displaced from their original radial and tangential rows, and at the same time they become distorted, elongated tangentially, flattened radially and in many cases crushed. Air spaces appear and disappear. The cell wall is thickened. The protoplasmic cell contents become indistinguishable and the cells become filled with a dense substance which may be continuous and coarsely granular or discontinuous and globular (Fig. 5). This differentiation of the hypodermis is a continuous process and cannot be divided into periods. Figs. 7 to 10 and the detailed description given below will indicate the rate at which this development takes place during the summer.

By the third week in June (Fig. 7) the enlargement of the ovary has resulted in many of the hypodermal cells becoming displaced. The original radial arrangement of the cells no longer exists in the two inner rows, but it still remains here and there in the two outer rows. The tangential arrangement may still be evident throughout, but it is frequently lost in the two inner rows. All the cells are considerably elongated tangentially and may reach a length of 35 μ . But although the cells have lengthened tangentially, they are not yet crushed. The small air spaces at the corners of the cells have largely disappeared, but large irregular spaces may be found among the cells of the two inner layers. These large "schizogenous" air spaces appear to have originated as a result of tissue distortion and cell displacement. This is of course brought about by the rapid enlargement of the young fruit. The cell wall has become much thicker, especially at the cell corners. The nucleus and cytoplasm are identified with difficulty, especially in cells which are filled with a heavy deposit, and the cells appear to be comparatively inactive. For a similar stage in the varieties studied by Tetley (4, p. 158), she reports that the cell division continues in these sub-epidermal layers. But very careful examination of the McIntosh material failed to reveal any indication of cell division in the hypodermis at this stage.

By the middle of July (Fig. 8) no new radical change has taken place, but all developments outlined for the third week in June are slightly more advanced. Owing, perhaps, to the heavy deposit in the cells, it is practically impossible to identify the protoplasmic cell contents, but the cell outlines may still be recognized everywhere. The changes in cell arrangement and cell shape have extended to the two outer layers just below the epidermis. The cells of these two outer layers are closely packed and without air spaces,

TABLE II

Season and illustrations	Hairs	Cuticle	The epidermis	The hypodermis
Middle of May	Each hair is living and continuous with and part of the epidermal cell from which it has developed. Collectively the hairs form a dense tangled mat with enclosed air spaces, thus providing a very efficient protective layer.	The cuticle is present but is so thin that it may be seen in a tangential section only.	Composed of small, active, thin-walled, isodiametric cells, that are dividing rapidly by radial walls.	Two layers of cells just under epidermis. Cells active and dividing by radial walls. Cell walls thicker than in either epidermis or cortical parenchyma. Cell shape and size similar to that of the cortical parenchyma.
Full bloom	The hairs are now slightly separated by the multiplication of the other epidermal cells. The cuticle is spreading over the side walls of the cell base within the epidermal layer. The cell contents are inconspicuous and as a living cell the hair is becoming inactive.	Continuous and about 1.4μ thick, but not uniform. It is thicker at the bases of the hairs and extends into the hair base and down the side walls within the epidermal layer.	Its cells are now elongated radially to twice their tangential width. They are still dividing and are still thin-walled and full of protoplasm.	Each cell has divided once tangentially; the layer is now four cells thick. Walls have thickened. Cell contents are dense. Nucleus and cytoplasm still evident. Small air spaces still observed at the corners of some cells.
Middle of June	The base is filled in with cuticle and the hairs are now dead. The distal portion is starting to drop off and thus this tissue is no longer effective as a protective layer.	About 5μ thick. It completely fills the hair base and is continuous except over the stomata or young lenticels where it shades off to nothing.	The cells are slightly broader, but the radial measurement is still greater than the tangential. The nucleus is evident, but the cytoplasm is becoming vacuolated. Cell division was not observed. That is the cells are still living but inactive.	Cells of the two inner layers displaced and tangentially elongated. Cell wall continuing to thicken. Cell contents so dense that nucleus can be identified in only a few cells. No indication of cell division. Small air spaces have disappeared from the outer layer; large air spaces are common throughout inner layers.
Middle of July	The hairs are now gone except in and around the depressions at the calyx and stalk ends.	About 11μ thick. It is now fairly uniform in thickness.	The cells have broadened and flattened so that the tangential measurement is now greater than the radial. It is difficult to distinguish protoplasmic contents. There is no trace of cell division, but accommodation to increase in size of young fruit is attained by the cells lengthening tangentially.	Changes in cell arrangement and cell shape have extended to outer layers. Protoplasmic contents indistinguishable. Cells of outer layer closely packed; air spaces throughout inner layers large and numerous. Heavy deposit present in most of cells. Cell walls very thick, especially at corners.

TABLE II—*Concluded*

Season and illustrations	Hairs	Cuticle	The epidermis	The hypodermis
Middle of August	Hairs gone.	About 16μ thick. On its inner surface it is starting to extend between the epidermal cells.	Its cells are starting to separate and the spaces between them are being filled by cuticle. The cell contents are disorganizing.	Cells and air spaces becoming tangentially elongated and radially flattened. Very heavy deposit in practically all cells. All changes in shape, etc., appear to be the result of pressure and stretching. Only vital activity evident is thickening of wall.
Harvest	Hairs gone.	Average thickness about 23μ , but thickness varies from 13 to 25μ . Extends between epidermal cells to hypodermis and even under epidermal cells.	Most of the cells are either flattened or crushed. Many of them are completely separated from adjacent cells by the cuticle, and some are even surrounded by this cuticular invasion. The cell contents have completely disintegrated.	All cells flattened and many crushed. At places exact cellular structure indistinguishable. Walls very thick and frequently laminated. Large air spaces practically eliminated. Most cells filled with dense deposit. No evidence of nucleus or cytoplasm. Layer as a whole very compact. Cells appear to be dead.

but large angular air spaces are evident everywhere throughout the two inner layers. There is still no indication of cell division and the cell walls have continued to thicken. Again it is at the corners that the thickening is most evident. In some of the cells the radial measurement has been reduced, apparently as the result of pressure.

By the middle of August (Fig. 9) the hypodermis starts to assume its permanent appearance. All regular arrangement is gone. All cells are elongated tangentially and flattened radially. Here and there a cell has been crushed. The air spaces are also tangentially elongated. The cell walls have become so thick that in cross section the hypodermis stands out sharply from the other tissues. Nearly all the cells are filled with a very heavy granular deposit and protoplasmic structures are quite indistinguishable. Certainly no cell division is occurring in the hypodermis at this stage and there are no gross morphological changes which could be attributed to the activity of individual cells. The whole hypodermal structure at this time appears to be completely under the influence of stresses and strains due to the enlargement of the fruit. The majority of the cells however are not crushed and they must be living to a certain degree, for the cell walls continue to thicken almost up to the time of harvest. A new feature at this stage is that here and there some of the outer cortical cells become involved in the crushing process and are incorporated as part of the hypodermal protective layer.

During the month of September the hypodermis reaches its final stage (Fig. 10). The cells have become so flattened and crushed, and the walls so thickened, that it is often impossible to identify the limits of an individual cell with any degree of accuracy. The air spaces are so crushed that it is difficult to tell whether certain indefinite tangential slits are crushed cells or crushed air spaces. At places the cell wall is very thick and laminated. The laminae always run tangentially. Again it is impossible to decide whether such a wall is a single, thick, laminated wall or two walls pressed together. No protoplasmic contents are observable and all the cells are filled with a dense deposit. Thus at this stage the hypodermis is a dense mass of closely packed, thick-walled, tangentially elongated, radially flattened or crushed cells. Probably most of these cells are either vitally inactive or dead. The thickness of this mature hypodermis varies from 50 to 100 μ .

To make this information regarding the structure and development of the protective layers more readily accessible, it has been summarized in tabular form. (See Table II.)

Acknowledgments

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CYLINDROCARPON EHRENBERGI WR., AND OTHER SPECIES, AS ROOT PARASITES OF ALFALFA AND SWEET CLOVER IN ALBERTA¹

By M. W. CORMACK²

Abstract

Cylindrocarpon Ehrenbergi appears to be one of the most important pathogenic fungi associated with early spring injury of roots of alfalfa and sweet clover in Alberta. It occurs in virgin and cultivated soils. It produces distinctive symptoms, and is highly pathogenic in the early spring, but less virulent during the growing season. It can invade unwounded roots through lenticels or the basal tissues of branch roots, or by direct penetration. It is also pathogenic on roots of *Trifolium* spp. This species has not been previously reported on the legume forage crops, and very little is known concerning its parasitism on other plants.

On the roots of alfalfa and sweet clover *C. obtusisporum* is slightly to moderately pathogenic, *C. radicicola* is very weakly pathogenic, and *C. olidum* is non-pathogenic. These species occur infrequently on diseased roots, and usually in association with *C. Ehrenbergi*. *C. radicicola* has been reported as an important root parasite of other plants.

Isolates of *C. Ehrenbergi* differ in degree of pathogenicity, and there is some evidence of host specialization. They also differ markedly in morphological and cultural characteristics, which, however, do not appear to be correlated with their parasitic abilities. The temperature range for growth of *C. Ehrenbergi* in pure culture is from -2° to 32° C., but different isolates do not have the same optima. Isolates with an optimum at about 19° C. caused the most damage in the early spring, while one which grew best at 24° C. proved the most virulent during summer. The optimum hydrogen ion concentration for growth of *C. Ehrenbergi* varies with the medium employed. Growth and spore germination studies indicate that the iso-electric point for the fungus lies at approximately pH 5.1.

Most of the commonly grown varieties of alfalfa and sweet clover are susceptible to attack by *C. Ehrenbergi*, but resistant species like *Medicago falcata* may prove valuable as plant breeding material. Apparently cereal crops are not attacked by the pathogen, therefore they should be grown for several years in severely infested fields.

During recent years, root rot of alfalfa and sweet clover has become increasingly prevalent in Alberta. Most of the damage occurs at the end of the dormant period when the plants are particularly susceptible to attack by certain pathogenic fungi. Later in the season the same or other fungi may cause a less destructive root rot of the growing plants.

Previous studies on the fungi associated with these root rots have been concerned with *Plenodomus Meliloti* and *Sclerotinia* sp. (23, 24). These commonly occurring pathogens are especially injurious in the early spring, and,

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as shown by Sanford (23), *P. Meliloti* ceases to attack the roots when growth starts. Further isolation and pathogenicity studies have shown that other fungi are involved. *Cylindrocarpon Ehrenbergi* was chosen for detailed study because of its wide distribution and frequent occurrence on diseased roots. The less commonly isolated species *C. obtusisporum*, *C. radicola* and *C. olidum* were included in the present investigation, which deals primarily with the taxonomy of the isolates, their pathology on roots of alfalfa and sweet clover, and certain aspects of their physiology.

Symptoms

C. Ehrenbergi usually produces characteristic symptoms on infected roots of alfalfa and sweet clover in the early spring. Periodic observations made on plants grown in artificially infested soil in the field have shown that infection begins at the first sign of thawing in the soil. The partially frozen soil is permeated with whitish mycelium, which rapidly invades the previously sound roots. This mycelium gradually becomes less evident and disappears when the soil becomes warm. Three representative stages of infection are shown in Fig. 1, A. The infected areas on the roots have first a water-soaked appearance, but soon increase in size, turn light brown in color, and finally dark brown. When infection is severe, the entire root system is often rotted within a week or two after the first sign of infection. Compact white masses of mycelium later form in the ruptured cork covering of the root, and develop into the characteristic sclerotia-like stromata of the fungus (Fig. 1, B and C; Plate I, D). These bodies are not uniform in color or consistency, but are usually salmon-orange in color and fairly hard and brittle.

The degree of infection with *C. Ehrenbergi* varies greatly from year to year, but sweet clover usually suffers more damage than alfalfa. By late May, severely attacked plants are dead and have roots which are either partially rotted (Fig. 1, B), or have been converted into decayed, misshapen masses (Fig. 1, C). The rotted tissues are more or less completely covered with an irregular layer of the aggregated stromata. At the same time, check plants, growing in adjacent plots of non-infested soil, are starting vigorous growth, and have sound roots (Fig. 1, B). When light infection occurs, irregular brown lesions of varying size are formed on the main tap or lateral roots. Stromata are usually aggregated near the centre of these lesions, or may be buried in the decayed tissues at the margin.

Crown rot is another type of injury frequently caused by *C. Ehrenbergi* in the early spring. Rotted areas of varying size appear at the crown, and involve all or part of the crown buds (Fig. 1, D). The plant dies when the crown buds are destroyed, but the root system may remain undecayed for some time.

During the growing season, natural infection of alfalfa and sweet clover roots by *C. Ehrenbergi* has seldom been observed. Likewise, the infection resulting from artificial inoculation of the roots is much less severe than that occurring in the early spring. The small, cinnamon-brown lesions produced

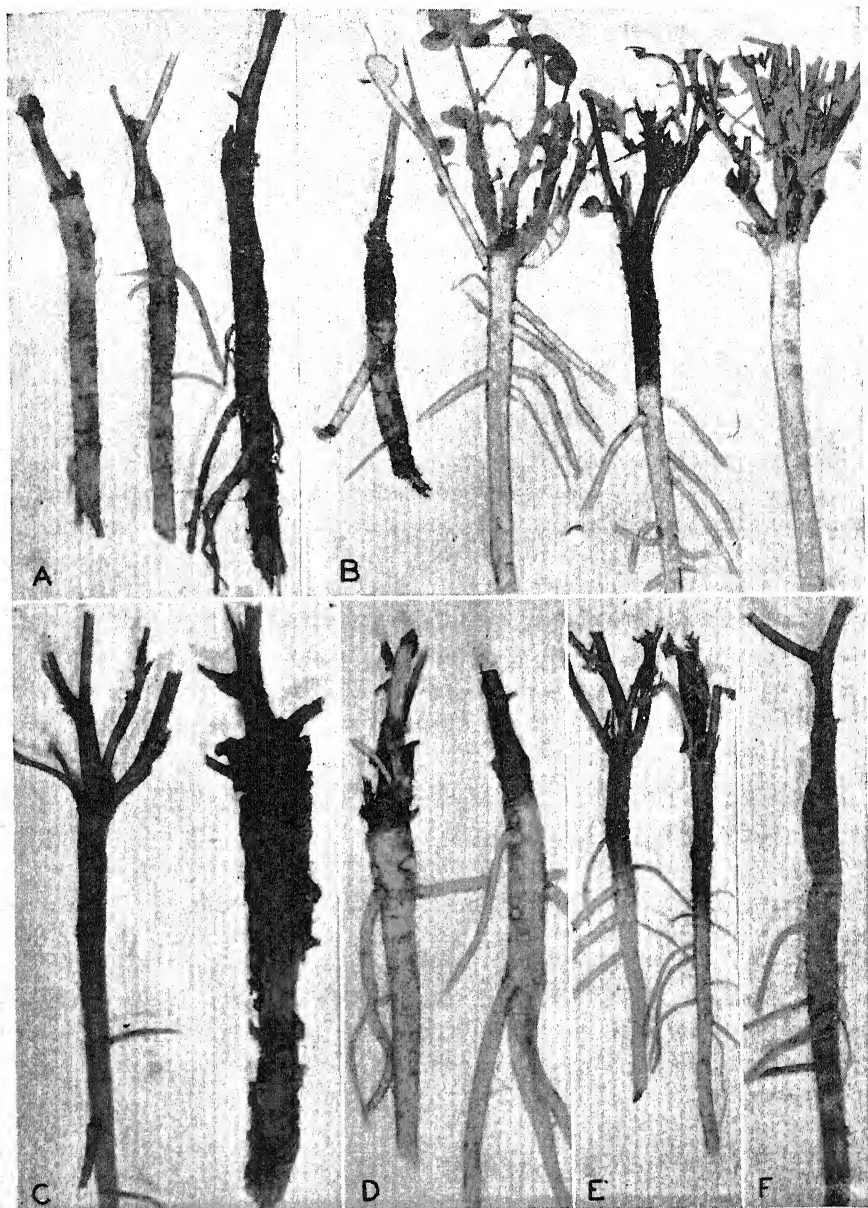


FIG. 1. A-D. Roots attacked in the early spring by *Cylindrocarpon Ehrenbergi*. A. Three stages of infection of sweet clover roots. B. Typical symptoms. Left, Arctic sweet clover, diseased and healthy; right, Grimm alfalfa, diseased and healthy. C. Severely attacked roots. Left, Grimm alfalfa; right, Arctic sweet clover. D. Crown infection of sweet clover. E. Early spring infection of alfalfa roots by *C. Ehrenbergi* (left), and by *C. obtusisporum* (right). F. Sweet clover root infected near the crown by *C. Ehrenbergi* and at the bottom by *Sclerotinia* sp.

on the roots of growing plants are slightly sunken toward the centre and have a narrow dark border. Stromata usually appear on the lesions within 30 days after inoculation.

C. obtusisporum seldom attacks inoculated roots as severely as does *C. Ehrenbergi*, and it does not produce stromata, but otherwise the symptoms are indistinguishable (Fig. 1, E). When *C. Ehrenbergi* does not form stromata on infected roots, the damage may be confused with that caused by other fungi in the early spring. There is usually no difficulty, however, in recognizing the pycnidia of *Plenodomus Meliloti* (23), or the pulpy rot and sclerotia produced by *Sclerotinia* sp. (8). Individual roots are sometimes attacked by more than one pathogen (Fig. 1, F). When mixed infection occurs, or distinctive symptoms are not produced, isolations are necessary to determine the fungi involved.

Isolation Studies

When infected roots are plated on agar, *Cylindrocarpon* spp. usually grow out very slowly, and are easily suppressed or overgrown by other fungi or bacteria. This made them difficult to isolate until the following methods were adopted. Pieces of diseased root tissue, cut from the interior margin of a lesion, were surface sterilized in mercuric chloride (1/1000) for two minutes, soaked in sterile distilled water for at least one hour, and plated on potato-dextrose agar. Incubation of the plates at a low temperature, as suggested by Bisby, Timonin, and James (7), also greatly facilitated the isolation of these fungi. More isolates were obtained from material incubated at 10° C. than at a higher or lower temperature.

The isolates were provisionally identified by means of the key and descriptions published by Wollenweber (29). Most of these determinations were confirmed by Dr. Wollenweber, who kindly examined representative cultures.

C. Ehrenbergi predominated among the isolates, and was obtained from diseased roots taken from 25 of the 45 fields examined during the past two years. It was isolated more frequently from alfalfa than from sweet clover, but occurred on both hosts at widely separated points in Alberta. These districts included Brooks and Lethbridge in the southern brown soil zone, Edmonton and Lacombe in the black soil zone, and Athabasca and Beaverlodge in the northern gray wooded and transition soil zones. The fungus also occurred on diseased sweet clover roots sent from Scott and Saskatoon, in Saskatchewan. In addition, it was isolated from diseased roots of red clover and from decaying pods of string bean.

C. obtusisporum, *C. olidum*, and *C. radicicola* were occasionally isolated from alfalfa roots, but only the last was obtained from sweet clover roots. These species occurred on a small proportion of the diseased root samples, usually in association with *C. Ehrenbergi*. *C. obtusisporum* was also isolated from diseased raspberry roots.

C. Ehrenbergi was readily isolated from the soil of several alfalfa fields by the dilution plate method. The other three species occurred less commonly in these soils. The presence of *C. Ehrenbergi* in virgin soil was also demon-

strated. Sound tap roots of alfalfa were surface sterilized before being buried, during October, in virgin prairie sod at Edmonton, Alberta, and at Rossburn, Manitoba. In the following spring some of the roots buried at each location bore stromata of *C. Ehrenbergi*, and the fungus was isolated.

Cylindrocarpon spp. were not isolated when seed of several varieties and samples of alfalfa and sweet clover was plated on agar.

Infection Studies

MATERIALS AND METHODS

Field-grown Grimm Alfalfa and Arctic sweet clover plants were used in all the general experiments, and were transplanted into boxes when required for greenhouse study. The field pathogenicity tests were made on dormant plants during the winter and on growing plants in the summer. In the winter experiments plants were inoculated in the late fall just prior to freeze-up. A small portion of oat hull inoculum was placed against each partially bared, but unwounded, tap root, after which the soil was replaced. Sterile oat hulls were placed against similar roots for checks, and all plants were left undisturbed until final notes were taken the following spring. A similar method was used in the summer experiments, except that the roots were wounded before inoculation, to promote infection. A thin flap of the outer tissue of the tap root was lifted with a sharp scalpel and a small fragment of mycelial inoculum was inserted beneath with a sterile wire hook. About four weeks later the plants were taken up.

In taking final notes each inoculated root was carefully examined and the degree of infection expressed by means of an arbitrary numerical rating on the following basis:

- 0 —No infection.
- 0.5—Slight trace of infection (surface tissues discolored).
- 1 —Trace infection (shallow lesions).
- 2-4—Light to medium infection (limited lesions of moderate depth).
- 5 —Medium infection (lesions extending about halfway through the tap root).
- 6-8—Medium to heavy infection (roots more than one-half rotted).
- 9 —Heavy infection (roots almost completely rotted).
- 10 —Plant dead.

PATHOGENICITY OF *Cylindrocarpon* spp.

Thirty-five isolates of *C. Ehrenbergi* and a few isolates of the other species were tested for pathogenicity on roots of alfalfa and sweet clover in several different field experiments. Results obtained with representative isolates in two winter tests (Table I), and two summer tests (Table II), show that *C. Ehrenbergi* is decidedly more pathogenic than the other species studied. *C. obtusisporum* is slightly to moderately pathogenic in the early spring, but it produces only a trace of infection during the summer. *C. radicola* is apparently only a weak wound parasite on roots of alfalfa and sweet clover, since

TABLE I

RELATIVE PATHOGENICITY OF SPECIES OF *Cylindrocarpon* AND ISOLATES OF *C. Ehrenbergi* ON ROOTS OF ALFALFA AND SWEET CLOVER. (WINTER TESTS 1935-36 AND 1936-37)

Species	Isolate		Alfalfa				Sweet Clover			
			Infection rating, %*			Rank†	Infection rating, %*			Rank†
	No.	Source	1935-36	1936-37	Av.		1935-36	1936-37	Av.	
<i>C. Ehrenbergi</i>	2	Alfalfa	24	5	14	10	22	23	22	13
<i>C. Ehrenbergi</i>	4	Sweet clover	18	5	11	11	61	60	60	5
<i>C. Ehrenbergi</i>	5	Sweet clover	35	16	25	6	33	91	62	4
<i>C. Ehrenbergi</i>	6	Alfalfa	47	49	48	1	12	73	42	9
<i>C. Ehrenbergi</i>	7	Alfalfa	23	27	25	6	37	40	38	12
<i>C. Ehrenbergi</i>	10	Sweet clover	18	47	32	5	30	91	60	5
<i>C. Ehrenbergi</i>	11	Sweet clover	17	27	22	8	64	92	78	1
<i>C. Ehrenbergi</i>	13	Bean pods	33	16	24	7	47	44	45	8
<i>C. Ehrenbergi</i>	14	Sweet clover	36	14	25	6	49	34	41	10
<i>C. Ehrenbergi</i>	15	Sweet clover	42	35	38	3	55	93	74	2
<i>C. Ehrenbergi</i>	18	Alfalfa	60	25	42	2	48	86	67	3
<i>C. Ehrenbergi</i>	22	Sweet clover	35	07	21	9	40	79	59	6
<i>C. Ehrenbergi</i>	23	Sweet clover	29	13	21	9	37	74	55	7
<i>C. Ehrenbergi</i>	28	Alfalfa	53	14	33	4	40	50	45	8
<i>C. Ehrenbergi</i>	30	Alfalfa	40	9	24	7	43	38	40	11
<i>C. obtusisporum</i>	26	Alfalfa	23	13	18		10	35	22	
<i>C. obtusisporum</i>	33	Alfalfa	31	11	21		17	10	13	
<i>C. radicola</i>	17	Sweet clover	13	6	9		10	11	10	
<i>C. radicola</i>	36	Alfalfa	14	5	9		1	12	6	
<i>C. olidum</i>	9	Alfalfa	7	2	4		0	0	0	
<i>C. olidum</i>	34	Alfalfa	5	6	5		0	0	0	
Check plants			6	1	3		0	0	0	

* Average numerical rating of 15 plants in each test.

† Rank of 15 isolates of *C. Ehrenbergi*, based on average infection rating for two years.

TABLE II

RELATIVE PATHOGENICITY OF SPECIES OF *Cylindrocarpon* AND ISOLATES OF *C. Ehrenbergi* ON ROOTS OF ALFALFA AND SWEET CLOVER. (SUMMER TESTS 1935 AND 1936)

Species	Isolate		Average infection rating, %					
			Alfalfa			Sweet clover		
	No.	Source	1935	1936	Av.	1935	1936	Av.
<i>C. Ehrenbergi</i>	2	Alfalfa	18	9	13	18	18	18
<i>C. Ehrenbergi</i>	4	Sweet clover	16	6	11	20	18	19
<i>C. Ehrenbergi</i>	5	Sweet clover	11	9	10	15	13	14
<i>C. Ehrenbergi</i>	6	Alfalfa	16	11	13	14	13	13
<i>C. Ehrenbergi</i>	7	Alfalfa	18	4	11	23	8	15
<i>C. Ehrenbergi</i>	10	Sweet clover	11	7	9	15	6	10
<i>C. Ehrenbergi</i>	11	Sweet clover	12	12	12	26	18	22
<i>C. Ehrenbergi</i>	13	Bean pods	10	11	11	8	12	10
<i>C. Ehrenbergi</i>	18	Alfalfa	12	10	11	11	2	6
<i>C. Ehrenbergi</i>	23	Sweet clover	14	13	13	25	26	25
<i>C. obtusisporum</i>	26	Alfalfa	10	17	13	8	12	10
<i>C. obtusisporum</i>	33	Alfalfa	5	7	6	8	9	8
<i>C. radicola</i>	17	Sweet clover	8	5	6	8	2	5
<i>C. radicola</i>	36	Alfalfa	6	2	4	2	0	1
<i>C. olidum</i>	9	Alfalfa	5	0	2	3	0	1
<i>C. olidum</i>	34	Alfalfa	7	0	3	3	1	2
Check plants			6	0	3	5	0	2

it seldom causes more than a slight discoloration of the tissues. None of the several isolates of *C. olidum* tested during the past three years have given definite evidence of pathogenicity.

PATHOGENICITY OF ISOLATES OF *C. Ehrenbergi*

It is evident from the data in Table I that isolates of *C. Ehrenbergi* differ in degree of pathogenicity. Some isolates are highly virulent on both alfalfa and sweet clover, while others produce relatively light infection. On the other hand, certain isolates which are highly pathogenic on sweet clover rank among those least pathogenic on alfalfa, and *vice versa*. Isolate 6, obtained from alfalfa, and Isolate 11, from sweet clover, are particularly virulent on their respective host plants, but otherwise there is no evidence of host specialization among the isolates. An isolate from bean pods attacked both hosts with moderate severity. Marked differences in pathogenicity are not evident in the summer tests (Table II), owing to the greatly reduced severity of infection.

VARIETAL AND HOST RANGE TESTS

Tests of varietal reaction to *C. Ehrenbergi* are still in a preliminary stage, but some indications of the potentialities of the commonly grown varieties of alfalfa and sweet clover have been obtained. In three winter tests (1934-35 to 1936-37), the soil was infested with the pathogen at seeding time, as in varietal tests with *Sclerotinia* sp. (24). Since this method gave a relatively low degree of infection with *C. Ehrenbergi*, roots of plants of each variety were also directly inoculated in the fall of 1936. The results obtained in these experiments are given in Table III.

TABLE III
REACTION OF VARIETIES OF ALFALFA AND SWEET CLOVER TO *Cylindrocarpon Ehrenbergi* IN WINTER TESTS

Variety	Average infection rating, %*				
	Soil infested				Roots inoculated
	1934-35	1935-36	1936-37	Av.	1936-37
Alfalfa					
<i>Medicago falcata</i>	1	3	4	3	11
Ladak	11	6	6	8	13
Grimm	7	14	4	8	21
Cossack	10	9	7	9	27
Baltic	11	9	6	9	20
Hardistan	20	17	8	15	30
Sweet clover					
Yellow blossom	22	16	18	19	69
Arctic	24	18	18	20	62
White blossom	28	14	19	20	55
Alpha No. 1	18	24	27	23	69
Zouave	27	26	22	25	65
Grundy County	31	29	21	27	57
Alborea	29	30	29	29	75

* Average infection rating of 60 plants of each variety in each test.

Medicago falcata (yellow-flowered alfalfa) appears to be relatively resistant to attack by *C. Ehrenbergi*. In previous studies (24), this species of alfalfa proved markedly resistant to attack by *Sclerotinia* sp. Hardistan alfalfa (*M. sativa*) had consistently the highest infection rating of the varieties tested. The variegated alfalfa varieties (*M. media*) gave a reaction intermediate between that of *M. falcata* and Hardistan.

All varieties of sweet clover tested were attacked with approximately equal severity by *C. Ehrenbergi*. Albotrea appeared slightly more susceptible than the other varieties, but further study is required. There were no clear-cut differences in reaction between varieties of *Melilotus alba* and *M. officinalis*, such as were obtained in studies with *Sclerotinia* sp. (24).

C. Ehrenbergi has also proved pathogenic on roots of *Trifolium* spp. In two winter tests it caused moderate to heavy infection on red clover, and light to moderate infection on alsike clover and white Dutch clover. At the same time *C. obtusisporum* produced light infection on the roots of these three hosts. A trace to light infection occurred, in all cases, under summer conditions.

C. Ehrenbergi caused slight rotting of wounded roots of turnip and carrot in a summer field experiment, but did not attack roots of beet or parsnip. It was also non-pathogenic to seedlings of wheat, oats and barley in the greenhouse.

FACTORS INFLUENCING INFECTION

Periodic observations on alfalfa and sweet clover roots inoculated with *C. Ehrenbergi* in the late fall showed that infection did not occur until the soil started to thaw out in April, but then proceeded very rapidly. However, relatively light infection occurred when the roots were inoculated shortly after the soil thawed out. In another experiment, dormant plants taken from partially frozen soil were inoculated, transplanted into boxes, and held at temperatures of 1.5°, 17°, and 21° C. Slightly greater infection was obtained at 1.5° C. than at the other temperatures, but in no case were the roots severely attacked. The plants were apparently past their most susceptible stage when the early spring inoculations were made. When the progress of infection during the spring was studied by inoculating plants at weekly intervals in the field, more infection occurred during April than later in the season.

Isolates of *C. Ehrenbergi* appear to vary with regard to the influence of temperature on infection. Isolate 23 produced light to moderate infection on roots of sweet clover growing in soil temperature tanks held at 18° and 21° C., but three other isolates were non-pathogenic at these temperatures. None of the isolates caused infection at temperatures of 24° and 27° C. Isolate 23 was also one of the most pathogenic of the isolates under the relatively high temperature conditions of summer (Table II), but one of the least pathogenic in the early spring (Table I).

Seedlings and young plants of alfalfa and sweet clover were less susceptible than older plants to attack by *C. Ehrenbergi*. In summer tests the infection rating for two-months-old plants was consistently less than for 14-months-old plants. With alfalfa, the roots of plants three and four years old were more severely attacked in the early spring than the roots of plants one and two years old.

Pathological Anatomy

The phenomena attending penetration and invasion of alfalfa and sweet clover roots by *C. Ehrenbergi* were studied for both early spring and summer infection. Roots were taken up for histological examination at various stages of disease development. Small pieces of each root were thoroughly fixed in modified Bouin's fluid, washed in four changes of 50% ethyl alcohol, and dehydrated in the tertiary butyl alcohol series described by Johansen (16). After infiltration with paraffin, the material was softened in water and cut, on a sliding microtome, into sections 12 μ thick. The Thionin-Orange G staining method described by Stoughton (25) gave very satisfactory differentiation of the fungus mycelium in the host tissues.

Wounding of the roots usually facilitates the entrance of *C. Ehrenbergi*, but is not necessary for successful infection. In the absence of wounds, the pathogen can enter the roots by three different avenues, namely: through the tissues at the base of branch roots, through lenticels, or by direct penetration of the cork covering. Infection occurs most commonly at the base of a branch root, which is a very vulnerable point, since it is protected only by a thin, and often broken layer of cork. The hyphae start to grow in the loose tissues around the base of a branch root, and soon rot it off and surge into the underlying tissues (Plate I, A). Lenticels are penetrated in a similar manner. Direct penetration appears to be less common, but has been frequently observed when a considerable mass of inoculum is in contact with the uninjured root. The hyphae mass up and push their way between the cork cells in an apparently mechanical manner (Plate I, B). The cork cells remain intact at first, but are soon ruptured and filled with the massed hyphae. This process is repeated in the deeper-lying cells, until the entire cork layer is penetrated. *C. Ehrenbergi* thus appears to effect penetration chiefly by mechanical means, in a manner similar to that described by Peltier and King (17) for direct penetration of alfalfa roots by *Ozonium omnivorum*. Previous studies on the invasion of alfalfa and sweet clover roots by *Sclerotinia* sp. and *Plenodomus Meliloti* (8) indicated that chemical action played a part in the penetration of cork layers by those pathogens.

After entering the root, the hyphae of *C. Ehrenbergi* develop rapidly in the phloem parenchyma. They progress singly, or more commonly in wefts or masses, through and between the cells and in all directions. Unless infection is severe, the hyphae are confined mainly to the parenchymatous tissues, and less commonly invade the phloem, cambium, and xylem. They seldom develop in the vessels. During summer, wound cork layers are often formed in advance of hyphal invasion, but, as with *Sclerotinia* sp. and *Plenodomus*

Meliloti (8), they do not appear effective in permanently checking the progress of the pathogen (Plate I, C). Hyphal advance ceases, however, in about 20 days after inoculation, under summer conditions. A distinct line of demarcation is formed between the diseased and healthy tissues. This border is of varying width and consists of disorganized cells filled with a dark-staining material. Apart from this, *C. Ehrenbergi* does not appear to exert any marked chemical action on the tissues in advance of hyphal invasion.

When infection has reached an advanced stage, *C. Ehrenbergi* produces stromata in irregular layers or sclerotia-like masses on the surface of the rotted tissues (Plate I, D). In the early stages of stromatal formation the hyphae mass up and fuse into small, soft bodies, which gradually increase in size and become fairly hard and brittle. Mature stromata are erumpent, or partly embedded in the disorganized cork layer, and they consist of closely packed hyphal elements united in a pseudo-parenchymatous tissue (Plate I, E).

The Pathogens

LITERATURE REVIEW OF THE GENUS *Cylindrocarpon*

Taxonomy. This relatively new genus was established by Wollenweber in 1913 (26). It belongs to the family Tuberculariaceae of the Fungi Imperfecti and contains fungi with cylindrical-clavate conidia, which were previously included in the genera *Fusarium*, *Ramularia*, *Fusidium*, *Fusisporium*, *Septocylindrium*, and *Atractium*. The species with known ascigerous stages are conidial forms of *Nectria* spp. Chlamydospore-producing forms originally placed in the genus *Ramularia* (26) were later transferred to *Cylindrocarpon* (27, 28). These two genera are distinguished by their manner of conidial abstriction (29). In *Cylindrocarpon*, as in *Fusarium*, conidia are formed in basipetal succession, with the oldest conidium uppermost. In *Ramularia*, however, each conidium arises acropetally as a bud-like outgrowth from an older conidium. Conidia of *Cylindrocarpon* differ from those of *Fusarium* in being cylindrical, less dorsiventral, and apedicellate, with a rounded apex. In many other respects the two genera are very similar.

Most of the species of *Cylindrocarpon* were fully described by Wollenweber (29) in 1928. They are differentiated on characteristics similar to those employed for *Fusarium*. The section *Ditissima* contains all the known conidial stages of members of the sub-genus *Coryneconnectria* and other species which do not form chlamydospores. Ten species and 11 varieties have been described in this section (18, 29). Species which produce chlamydospores are placed in the section *Chlamydospora*, which now contains 11 species and one variety (18, 29, 30). Members of this section are also characterized by relatively small, usually straight conidia, which often have basal papillae. The ascigerous stages belong to the sub-genus *Neonectriae*, but are only known for two of the species.

The four species studied in the present investigation belong to the section *Chlamydospora*. *C. Ehrenbergi* Wr. was first described as *Fusarium candidum*

by Ehrenberg in 1818. Other synonyms are *Ramularia candida* (Ehr.) Wr., and *Fusarium uniseptatum* v. Hoehnel. The ascigerous stage, *Neonectria caespitosae* (Fuck.) Wr., which was found on bark of *Ulmus* and dead roots of *Betula* in Germany (29), has not been observed during the present study.

Synonyms for the other species studied are as follows:

C. obtusisporum (Cke. & Hark.) Wr.—*Fusarium obtusisporum* Cke. & Hark., *Ramularia obtusispora* (Cke. & Hark.) Wr., *R. anchlussae* Wr.

C. radiculicola Wr.—*Fusarium polymorphum* Marchal, *Ramularia macrospora* Wr.

C. olidum Wr.—*Fusarium solani* Sacc., *Ramularia olida* Wr.

Distribution and Parasitism of the Species. Species of *Cylindrocarpon* have been isolated from decaying roots, tubers, bulbs, stems, branches, and fruit of many plants (29). Those of most importance on the aboveground parts of plants are the conidial stages of *Nectria* spp. European canker of pears, apples, and other trees, caused by *N. galligena* (*C. Mali*), is a destructive disease in many countries (2, 33). Other canker-producing species were studied by Richter (20), in Europe, and Zeller (34), in Oregon. Ehrlich (10) made a comprehensive study of the beech bark disease caused by *N. coccineae* (*C. candidum*). Wollenweber and Hochapfel (32) recently found that several species of *Cylindrocarpon* occurring on rotted fruits were parasitic on fruits of apple, pear and tomato.

Species of the section *Chlamydospora* occur quite commonly on the underground parts of plants (29), but their parasitism has received relatively little attention. One of the best known species, *C. radiculicola*, causes a destructive bulb rot and root rot of daffodils, narcissus, hyacinth, cyclamen, and other bulbous plants in England and Europe (3, 11, 31). Berkeley and Lauder-Thomson (5) found this species to be the most virulent of the fungi isolated from the "black lesion" type of strawberry root rot in England. It has been also found associated with the root-rot complexes of strawberry and raspberry in Ontario (4, 13). Jenkins (15) reported that *Ramularia macrospora* (*C. radiculicola*) caused a disease resembling crown canker on greenhouse roses in the United States. Hodges (14) isolated *C. radiculicola*, *C. radiculicola* var. *violaceum*, and *C. didymum* from sugar beets, but found that they caused little or no damage to that host. Root rot of ginseng in Ontario, studied by Hildebrand (12), was caused by *Cylindrocarpon*-like fungi which were referred to the genus *Ramularia*.

The occurrence of *Cylindrocarpon* spp. in soils is further evidence of their wide distribution. Wollenweber (29) reported *C. radiculicola*, *C. didymum* and *C. Magnusianum* from soil. Bisby, James and Timonin (6) isolated the following forms from Manitoba soils: *C. macrosporum*, *C. candidum*, *C. candidum* var. *majus*, *C. heteronemum*, and *C. didymum*. In a later study (7), these workers found the above species of fairly common occurrence, especially in the sub-surface horizons of the soil. Reinking (19) recently found *C. olidum*

and two new varieties of this species, also *C. curvatum*, and *C. radiculicola* in soils of banana plantations in South America. The relative prevalence of these species varied at different depths in the soil.

Cylindrocarpon spp. have apparently not been previously reported on alfalfa, sweet clover, or other legume forage crops. The four species isolated from these hosts during the present investigation have been incidentally reported on various plants, but, with the exception of *C. radiculicola*, discussed above, little is known concerning their parasitism. *C. Ehrenbergi* was mentioned by Wollenweber (26) as a possible wound parasite on partially decayed roots of carrots and other plants. Later (29), he listed it as occurring in Europe on roots of *Daucus*; tubers of *Solanum tuberosum*; bark of *Fagus*; branches of *Betula*; stalks of *Brassica*, *Helianthus*, and *Lupinus*; fruits of *Aesculus*, *Juglans*, and *Pirus*; and seedlings of *Pinus*. *C. obtusisporum* has been found on rotting seed of *Triticum vulgare*, *Phytophthora*-rotted potato tubers, branches of *Tilia* and *Acaciae*, bark of *Pirus*, and wood of *Ribes* (29). *C. olidum* was reported by Wollenweber (29) on decayed potato tubers in Europe, and by Anliker (1) on diseased rye seedlings in Switzerland.

DESCRIPTION OF THE ISOLATES

General Characteristics. The isolates of *C. Ehrenbergi* obtained from roots of alfalfa and sweet clover correspond fairly closely to Wollenweber's Latin diagnosis of the species (29). Most strains produce white mycelium in a compact, cottony, slow-growing colony, which is white, or some shade of orange, salmon, or vinaceous, depending upon the color of the basal stromatal layer (Plate I, F). Characteristic sclerotia-like stromata, resembling those formed on infected roots (Plate I, D), are often produced on agar. They are scattered throughout the colony, or aggregated in masses or concentric rings, and vary in color from yellow, orange, salmon, cinnamon to brown, with occasionally an olive-green tinge. Conidia are usually absent, or sparingly borne on the mycelium, in cultures less than one month old. They are produced successively at the tip of a conidiophore, and often adhere, side by side, to form a false head. In older cultures, creamy white shiny masses or layers of conidia are borne in sporodochia or pionnotes which develop from stromata or on the mycelium. The conidia are cylindrical and straight, or slightly curved, with ellipsoidal to conical ends (Fig. 2, A-B). They are typically one-septate, with an average size range of $20\text{--}27 \times 3.3\text{--}3.9\mu$, in the strains studied (Table IV). Pluri-septate conidia were not observed and non-septate forms were rare in most of the cultures. Chlamydospores are usually scarce. They are spherical to ovate, intercalary, single or in short chains, and average about $10 \times 8\mu$ in size (Fig. 2, C).

Isolates of *C. obtusisporum* can be readily distinguished from those of *C. Ehrenbergi*. Short, dense, white or pale yellow mycelium is produced on a dark brown stromatal layer, forming a compact, zoned, yellowish-brown colony (Plate I, F). Cylindrical-clavate, 0- to 3-septate conidia, with obtuse-conical ends (Fig. 2, D), are borne on the mycelium and, more rarely,

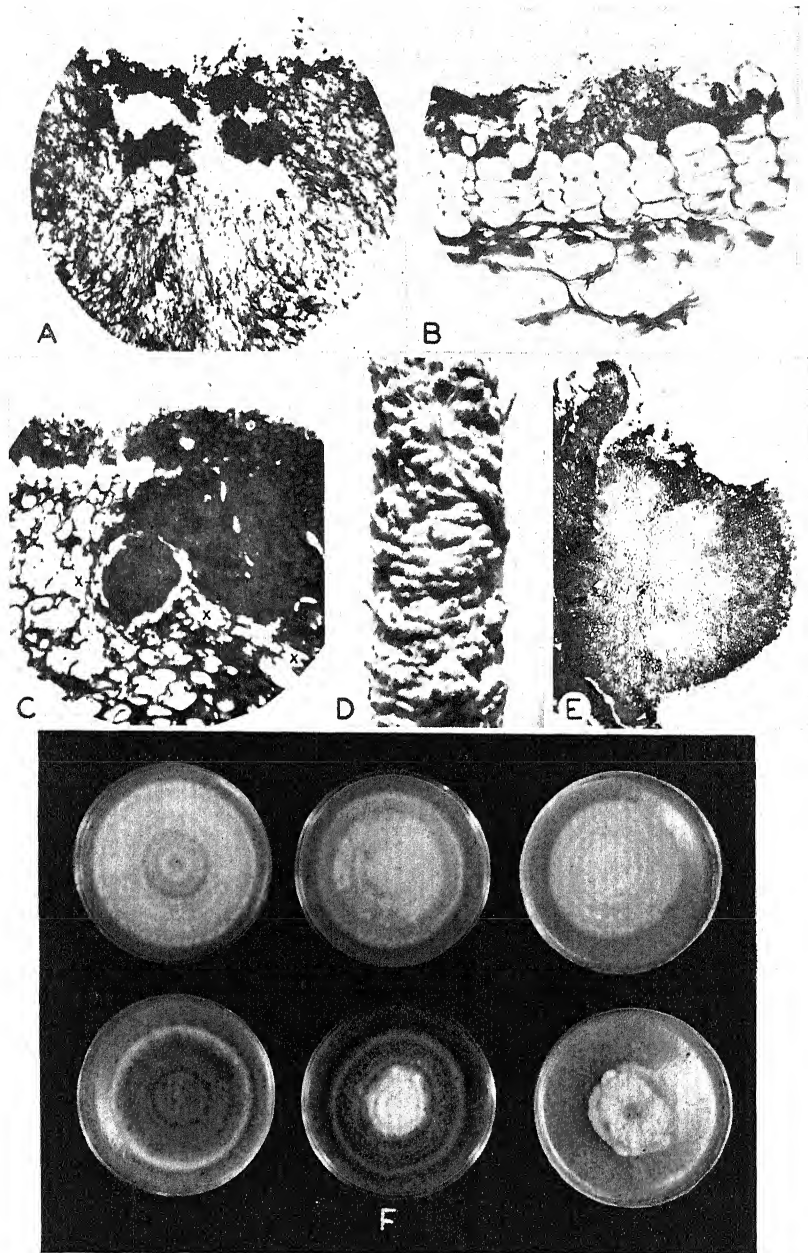


PLATE I. A-E. Invasion and development of *Cylindrocarpon Ehrenbergi* in roots of alfalfa and sweet clover. A. Hyphae entering a tap root through the base of a branch root which has been rotted off. $\times 85$. B. Massed hyphae effecting direct penetration by pushing between the cork cells of the outer protective layer of the root. $\times 330$. C. Ineffective wound cork layers (X) formed around the invading hyphal mass. $\times 130$. D. Numerous stromata aggregated on the surface of an infected root. $\times 3$. E. Longitudinal section of a single stroma which is partly embedded in a diseased root. $\times 85$. F. Ten-day-old colonies of *Cylindrocarpon* spp. on potato-dextrose agar. Left to right: Top row, *C. Ehrenbergi*. Isolates 7, 4, and 30. Bottom row: *C. obtusisporum*, *C. radicicola*, and *C. olidum*.

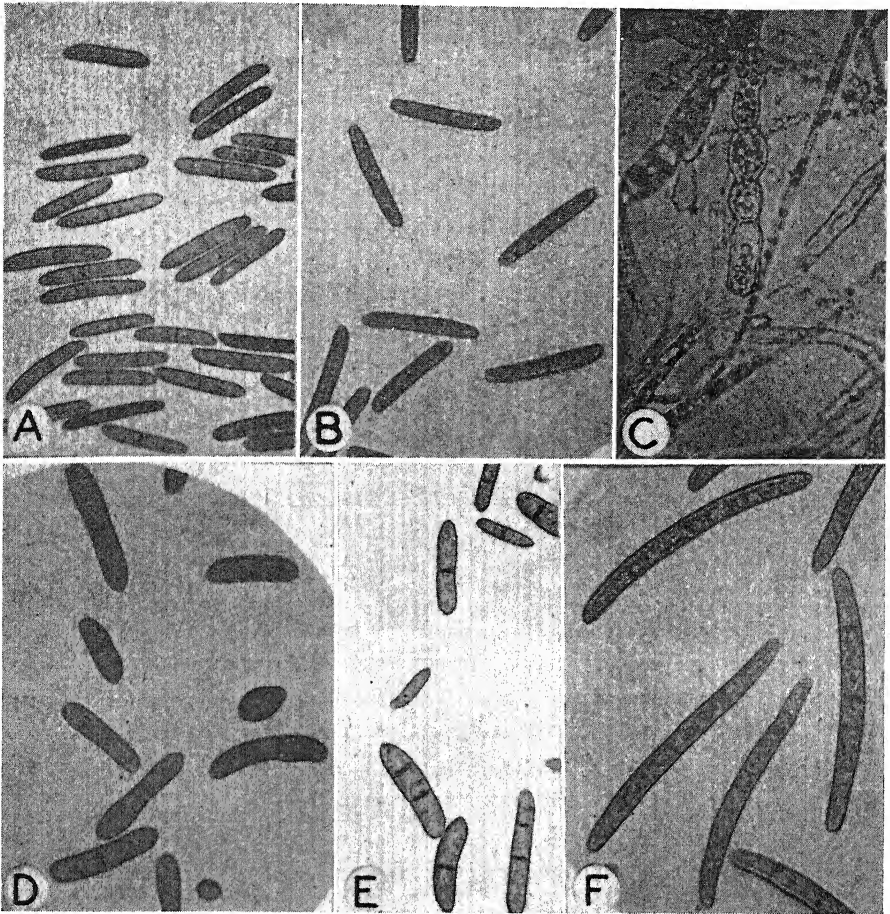


FIG. 2. Conidia and chlamydospores of *Cylindrocarpon* spp. from six-weeks-old colonies grown on potato dextrose agar. $\times 475$. (Lightly stained.) A-C. *C. Ehrenbergi*. Conidia of Isolates 4 and 6, and chlamydospores of Isolate 11, respectively. D-F. Conidia of *C. obtusisporum*, *C. radiculicola*, and *C. olidum*, respectively.

in sporodochia or pionnotes. The size of the prevailing 0- and 1-septate forms (150 of each measured), in six-weeks-old cultures on potato-dextrose agar, was as follows:

0-septate, average $12.9 \times 4.7\mu$; majority $9.1-18.2 \times 4.2-5.7\mu$; total range $7.6-19.8 \times 3.4-7.0\mu$.

1-septate, average $21.1 \times 5.0\mu$; majority $15.2-27.4 \times 4.6-5.7\mu$; total range $9.8-33.4 \times 4.2-6.1\mu$.

Chlamydospores are rare and not well differentiated from bulbous swellings on the mycelium.

C. radiculicola has relatively sparse, pale yellow to light brown aerial mycelium produced on a stromatal layer which sometimes has a purplish tinge (Plate I,F).

TABLE IV
CHARACTERISTICS OF GROUPED ISOLATES OF *Cylindrocarpon Ehrenbergi*

Group	No. of isolates	Colony		Conidia			Stromata	Chlamydo-spores
		Growth rate	Color	Production	Type	Av. size, (μ)		
A	5	Slow	Vinaceous to ferruginous	Abundant	Long, slender, 1-sept.	25-27 \times 3.4	Scarce	Scarce
B	10	Medium	Varied but mostly salmon	Scarce	Fairly short 1-sept.	21-24 \times 3.4-3.9	Abundant	Scarce
C	3	Medium	White to vinaceous	Medium	Short, 1-sept.	18-22 \times 3.4-3.6	Medium	Scarce
D	7	Rapid	Pinkish cinnamon	Abundant	Variable, 0 and 1-sept.	20-22 \times 3.3-3.7	Variable	Scarce
E	2	Medium	White	Scarce	Fairly short 1-sept.	20-23 \times 3.4-3.5	Absent	Scarce
F	1	Medium	Cinnamon	Scarce	Short 1-sept.	21.7 \times 3.5	Medium	Abundant

The conidia, formed on the mycelium or in pinnules, are cylindrical, mostly 1- to 2-septate, and straight or slightly curved, with ellipsoidal ends (Fig. 2, E). In six-weeks-old cultures on potato-dextrose agar, 150 of the predominant 1-septate conidia measured as follows: average $23.4 \times 5.4\mu$; majority $18.2-28.9 \times 4.6-6.1\mu$; total range $13.7-31.9 \times 3.8-6.5\mu$. Chlamydo-spores are abundantly produced. They are brown, nearly spherical, intercalary, and average about 11μ in diameter.

C. olidum is most readily recognized by the strong earthy odor produced in pure culture. The abundant, white to light brown mycelium has raised patches and tufts, formed in an irregular zonal manner, on a light brown stromatal layer (Plate I, F). Conidia are produced in white to honey-colored sporodochial masses. They are large, distinctly curved, and mostly 3-septate, with ellipsoidal ends (Fig. 2, F). In six-weeks-old cultures on potato-dextrose agar, 150 three-septate conidia of one isolate measured as follows: average $54.6 \times 6.1\mu$; majority $48.6-60.8 \times 5.7-6.5\mu$; total range $38.6-68.3 \times 4.6-6.8\mu$. Other isolates have slightly smaller or larger conidia. The fairly numerous chlamydo-spores are hyaline, 1- to 2-celled, verrucose, mostly intercalary, and average about $12 \times 7\mu$ in size.

Description of C. Ehrenbergi Strains. Isolates of *C. Ehrenbergi* were found to differ greatly in morphological, cultural, and physiological characteristics, as well as in pathogenicity. A detailed study was made of 28 isolates in an attempt to determine their range of variability. These isolates have been grouped in Table IV for purposes of comparison.

Some isolates grow much more rapidly than others at room temperature (Plate I, F), but the differences are less marked at low temperatures. Color of the mycelium and substratum varies greatly in different strains, and is also influenced by the kind of medium, temperature and other conditions. The colony colors listed in Table IV are based on Ridgway (21), and are those which predominated under a wide range of conditions. Marked and consistent differences occur in the rapidity and abundance of conidial formation. Some isolates (Groups A and D) produce creamy white masses of conidia in four to six weeks, while other isolates (Group B) never produce them in masses, and only sparingly on the mycelium. These isolates also differ greatly in the number and type of stromata produced.

Another striking point of difference between isolates is in conidial size and shape. The range of size for representative isolates, grown under identical conditions, is given in Table V. There is a marked contrast between the long, relatively slender conidia of isolates in Group A and the short conidia

TABLE V
SIZE IN MICRONS OF CONIDIA FROM DIFFERENT ISOLATES OF
*Cylindrocarpon Ehrenbergi**

Group	Isolate No.	Type of spore	Average size	Size of majority (90%)	Total range
A	4	1-sept.	26.0×3.4	23.5-29.7×3.0-3.4	20.6-32.7×2.6-3.7
	14	1-sept.	27.0×3.4	24.1-29.2×3.0-3.4	21.0-32.7×3.0-3.8
B	6	1-sept.	22.0×3.4	19.8-24.1×3.0-3.8	17.2-25.8×3.0-4.3
	18	1-sept.	21.2×3.9	20.6-23.3×3.8-4.3	18.9-24.1×3.4-4.7
C	10	1-sept.	21.0×3.4	18.9-24.1×3.4-3.8	16.4-25.0×3.0-4.3
D	23	1-sept.	22.1×3.3	20.6-24.1×3.0-3.4	17.2-25.8×3.0-3.8
	20	1-sept.	20.3×3.7	17.2-23.3×3.4-3.8	14.7-25.0×3.0-4.3
	20	0-sept.	11.2×3.2	9.1-12.9×3.0-3.4	7.8-15.2×2.6-4.3
F	11	1-sept.	22.1×3.3	20.6-24.1×3.0-3.4	17.2-25.8×3.0-3.8
	11	Chlamydospores	10.6×7.8	8.6-12.0×6.9-9.5	6.9-15.5×5.2-13.8

* Measurement of 150 spores of each isolate from two-months-old cultures on potato dextrose agar.

of isolates in Group B (Fig. 2, A-B). The former type is consistently associated with the slow-growing, dark-colored colony characteristic of Group A. Isolates in the other groups are more variable and, in general, do not show any correlation between morphological and cultural characteristics.

Certain isolates possess distinctive characteristics not found in any of the other strains studied. For example, Isolate 20 produces approximately 33% small, non-septate conidia, which usually represent less than 1% of the conidia of other isolates. Chlamydospores are sparingly produced by most isolates, but occur abundantly under all conditions in cultures of Isolate 11. Two isolates (Group E) produce white sparse mycelium, and no stromata (Plate I, F),

but otherwise resemble the isolates of Group B. This type may possibly arise as a variant in nature, since it occasionally appears as a sector in pure culture.

There is apparently no correlation between the morphological and cultural characteristics of an isolate and its virulence, for isolates in each of the groups described above were among those most pathogenic on alfalfa, and also among those most pathogenic on sweet clover (Table I).

CULTURAL STUDIES

Influence of Nutrients. Potato-dextrose agar supported good growth of *Cylindrocarpum* spp., and was used in all the general experiments. *C. Ehrenbergi* was tested on a variety of natural and synthetic media. Potato-dextrose agar gave the best results, but the fungus also grew well on Czapek's synthetic malt extract, and soil extract agars, and on agar media prepared from the expressed root juices of alfalfa and sweet clover. Media unsuitable for mycelial growth were corn meal, bean pod, prune, oat hull, Dox's inorganic salt, and Molisch's salt peptone agars.

Dextrose and peptone were added in varying concentrations to agar media. Moderate amounts of dextrose stimulated mycelial growth of *C. Ehrenbergi*, and usually favored stromatal production. Conidial formation was definitely retarded on media containing more than 2% dextrose. Peptone stimulated mycelial growth for the first few days, but hastened staling action in later growth. It had a slight stimulating effect on stromatal and conidial formation. Other studies also indicated the importance of the influence of the C/N ratio on growth and reproduction of the fungus.

On ordinary media most strains of *C. Ehrenbergi* produce very few stromata or conidia until the cultures are at least one month old. A modified Molisch's salt-peptone agar, however, induced good stromatal and conidial formation within 10 to 15 days. Stromata and conidia also formed quite rapidly on steam-sterilized pieces of alfalfa root in test tubes. Similarly prepared pieces of roots of sweet clover, red clover, and alsike clover did not provide favorable media for their development.

Influence of Temperature. The relation of temperature to growth in pure culture of *C. Ehrenbergi* and *C. obtusisporum* was studied in several different experiments. Uniform circular pieces of inoculum were transferred to plates of potato-dextrose agar, which were immediately incubated in quadruplicate at controlled temperatures ranging from 1° to 33° C. Representative results obtained with five isolates of *C. Ehrenbergi* and one isolate of *C. obtusisporum* are given in Table VI.

All five isolates of *C. Ehrenbergi* grew well at temperatures ranging from 14° to 27° C., and were inhibited, but not killed, at 33° C. Isolates 4, 20, and 31, however, grew best at about 20° C., while Isolates 11 and 23 had a somewhat higher optimum temperature. These temperature relations were

TABLE VI

GROWTH OF FIVE ISOLATES OF *Cylindrocarpon Ehrenbergi* AND ONE ISOLATE OF *C. obtusisporum* AFTER INCUBATION FOR SEVEN DAYS AT TEMPERATURES RANGING FROM 1° TO 33° C.

Species	Isolate No.	Average diameter of colonies in mm. at different temperatures									
		1°	5°	9°	14°	17°	20°	24°	27°	30°	33°
<i>C. Ehrenbergi</i>	4	5	8	12	19	21	23	22	20	8	0
<i>C. Ehrenbergi</i>	20	5	10	17	30	35	38	37	19	11	0
<i>C. Ehrenbergi</i>	31	5	10	14	23	31	32	27	20	6	0
<i>C. Ehrenbergi</i>	11	5	9	15	26	30	32	33	18	7	0
<i>C. Ehrenbergi</i>	23	5	10	15	21	26	29	32	29	11	0
<i>C. obtusisporum</i>	26	0	5	10	25	31	31	16	13	0	0

confirmed in another experiment with some of the isolates (Fig. 3). The relatively high temperature relation of Isolate 23 may explain its ability, during summer, to attack roots of alfalfa and sweet clover more severely than most other isolates (Table II).

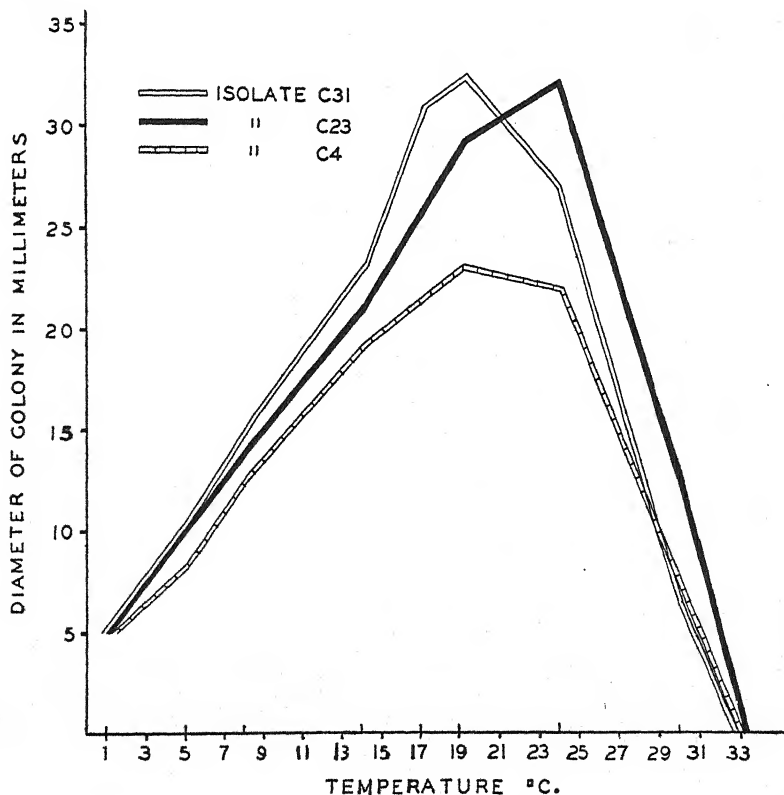


FIG. 3. Influence of temperature on the growth of three isolates of *Cylindrocarpon Ehrenbergi* on potato-dextrose agar. Incubated seven days.

C. obtusisporum had a relatively narrow temperature range for growth, with an optimum at 17° to 20° C. It was greatly retarded at the higher temperatures, and failed to grow at 30° C.

The low-temperature relations of the isolates were of special interest in this investigation, owing to their association with winter injury of alfalfa and sweet clover. *C. Ehrenbergi* started growth on frozen agar three weeks after freshly transferred cultures of several isolates had been placed in a freezing chamber at -2° C. Isolates of *C. obtusisporum*, *C. radicola*, and *C. olidum* did not grow at this temperature. At 1° and 5° C. all isolates of *C. Ehrenbergi* developed at an approximately equal rate (Table VI). At 1° C. *C. obtusisporum* and *C. radicola* started growth in 10 and 14 days, respectively. *C. olidum* failed to grow at 1° C., and developed very slowly at temperatures below 10° C.

Influence of Hydrogen Ion Concentration. Isolates of *C. Ehrenbergi* were grown on buffered potato-dextrose and Czapek's liquid and agar media adjusted to a wide range of hydrogen ion concentration. A large batch of each medium was made up with less than the normal amount of water, and divided into lots for adjustment. Each lot was sterilized and then adjusted to the required reaction by adding sterilized hydrochloric acid or sodium hydroxide. If required, sterile water was added to bring each lot up to normal concentration. pH determinations were made with a potentiometer and quinhydrone, and were checked by the colorimetric method. The agar media were held at 40° C. for adjustment and determination of pH, after which uniform amounts were poured into sterile plates. With the solutions, each lot was allowed to stand for 24 hr. before final adjustment, and then 50-cc. portions were pipetted into sterile flasks. Uniform pieces of inoculum were transferred to the plates and flasks, which were incubated at room temperature. Representative results obtained with one isolate are given in Table VII.

TABLE VII

INFLUENCE OF THE HYDROGEN ION CONCENTRATION OF AGAR AND LIQUID MEDIA ON GROWTH OF
Cylindrocarpum Ehrenbergi (Isolate 4)

Potato dextrose agar		Czapek's agar		Czapek's solution			
Initial pH	Diameter* of colonies	Initial pH	Diameter* of colonies	Initial pH	Final pH	Final pH of control	Dry weight of mycelium†
2.1	0						
3.1	0	2.8	8	3.0	3.1	3.0	0
4.1	22	4.0	28	3.9	4.6	3.8	75
4.7	27	4.6	29	4.6	5.2	4.5	154
5.2	30	5.1	28	5.2	5.2	5.2	125
5.9	31	5.8	27	5.8	5.4	5.8	127
6.3	31	6.2	29	6.4	6.0	6.4	129
7.0	31	6.9	28	7.0	6.2	6.9	133
7.6	31	7.7	25	7.8	6.6	7.6	262
8.4	30	8.4	24	8.4	7.0	8.2	183
9.4	26	9.5	21	9.7	7.8	9.5	66

* Average diameter, in millimeters, of four colonies, after seven days' incubation.

† Average dry weight, in milligrams, of four colonies, after two weeks' incubation.

C. Ehrenbergi grew well at hydrogen ion concentrations ranging from 4.0 to 9.5. The optimum reaction for growth varied with the medium employed. On potato-dextrose agar, the best growth occurred at pH 5.9 to 7.6, and there was none at pH values lower than 4.1. The optimum was also poorly defined on Czapek's agar, but the fungus was more tolerant to the acid reactions and grew at pH 2.8. More conclusive results were obtained in Czapek's solution, where two distinct optimum points for growth occurred at pH values of 4.6 and 7.8. Less growth at pH 5.2 to 6.4 indicated an isoelectric point in this region (22). Growth of the fungus resulted in the medium becoming more alkaline when the initial pH values were below 5.2, and more acid when the initial values were above this point. Only slight pH changes occurred in the corresponding control solutions where the fungus was not grown.

Spore Germination of *C. Ehrenbergi*

Conidia of *C. Ehrenbergi* were germinated at different temperatures and in solutions of varying hydrogen ion concentration. Three drops of a uniform suspension of mature conidia were distributed on a chemically clean, sterile, microscope slide. The slide was placed on a piece of bent glass tubing in an inverted Petri dish, which was sealed with water. Under favorable conditions, the conidia germinated within 15 hr., by producing a germ tube at the end of each cell. The percentage of spore germination for each treatment was calculated from a count of approximately 100 spores in each of the triplicate drops.

Spore suspensions of Isolate 4 in potato-dextrose solution (pH 7.0) were incubated at temperatures ranging from 2° to 33° C. In 15 hr., 97% of the conidia had germinated at 20° C., 93% at 17° C., 75% at 13° and 25° C., and 52% at 28° C. Germination was inhibited at higher temperatures. At low temperatures the spores germinated slowly, but even at 2° C. most of them produced germ tubes within four days.

Conidia of Isolate 4 were germinated in potato-dextrose solutions with pH values ranging from 2.0 to 8.6. The results obtained with duplicate slides incubated at 18° and 24° C. are given in the first columns of Table VIII. The spores germinated readily at pH values ranging from 3.4 to 8.6. At the lower pH values, higher germination occurred at 18° C., than at 24° C. It started in four days at pH 3.0 and 2.4, but was inhibited at pH 2.0.

At both temperatures, optimum points for spore germination occurred at pH 4.0 to 4.4, and at pH 7.2 to 7.6, with a distinct minimum at pH 5.2. This minimum point was more marked at 18° than at 24° C. It was more accurately determined in a second experiment where solutions with a narrower range of pH values were employed (Table VIII). The minimum or apparent isoelectric point for spore germination occurred at pH 5.1, as evidenced by a lower germination and by the relatively weak germ tube growth of conidia which had germinated. Similar results were obtained with other isolates. Since a similar, but less well defined, minimum point occurred in the growth studies, it is concluded that the isoelectric point for *C. Ehrenbergi* lies at approximately pH 5.1.

TABLE VIII

INFLUENCE OF HYDROGEN ION CONCENTRATION AND TEMPERATURE ON SPORE GERMINATION OF
Cylindrocarpon Ehrenbergi IN POTATO DEXTROSE SOLUTION

First experiment			Second experiment		
Initial pH	Per cent germination*		Initial pH	Per cent germination, 20° C.	Germ tube† length, 20° C.
	18° C.	24° C.			
2.0	0	0			
2.4	0	0			
3.0	0	0	4.7	97	
3.4	84	46	4.8	95	
4.0	100	99	4.9	88	18.6
4.4	99	98	5.0	75	9.8
4.8	74	87	5.1	57	3.5
5.2	28	70	5.2	89	12.0
5.6	85	94	5.3	92	22.1
6.0	86	96	5.4	89	
6.4	82	96			
6.8	75	98			
7.2	94	100			
7.6	96	98			
8.0	88	93			
8.6	87	90			

* Per cent germination in 15 hr.

† Average length in microns of 100 germ tubes in 15 hr.

Discussion

The foregoing results indicate that *C. Ehrenbergi* is probably one of the most important of the fungi associated with early spring injury of alfalfa and sweet clover in Alberta. In fact, it may be even more prevalent than *Plenodomus Meliloti* and *Sclerotinia* sp. It has been isolated from a large proportion of the diseased root samples collected from widely separated points throughout the province, and all isolates studied have proved pathogenic on roots of alfalfa and sweet clover. Furthermore, the fungus can also attack roots of *Trifolium* spp. It has not been previously reported on any of the legume forage crops, and practically nothing is known concerning its parasitism on other plants. However, Wollenweber (29) reported it as occurring in Europe on decayed roots and other parts of various plants, and it has been found in both cultivated and virgin soils during the present study. Hence, it seems possible that future studies may reveal *C. Ehrenbergi* as an important root parasite of other plants.

Other species of *Cylindrocarpon* are apparently of incidental occurrence as saprophytes or weak parasites on roots of alfalfa and sweet clover, since they have been isolated infrequently, and usually in association with *C. Ehrenbergi*. However, *C. obtusisporum* can cause moderate damage to the roots, and may yet prove of importance. *C. radicola* has proved weakly parasitic on roots of alfalfa and sweet clover, despite the fact that this species has been reported as a destructive parasite on the roots and bulbs of various other plants. *C. olidum* was the only species studied which showed no evidence of parasitism on the legumes.

The relation of temperature to growth of *C. Ehrenbergi* has proved of special significance in this study. In the first place, the ability of the isolates to grow at a temperature at or slightly below freezing probably explains why they can attack and cause serious damage to roots of alfalfa and sweet clover in the early spring. Also, the isolate that was most pathogenic in the summer, and least virulent in the early spring, grew best at about 24° C., while those causing least injury in summer and maximum damage the following winter belonged to a group having a lower optimum for growth. Hence, these results indicate that certain races of *C. Ehrenbergi* may be chiefly responsible for the damage occurring in the early spring, while other races with a higher temperature relation may attack the roots of growing plants later.

With regard to control measures for *C. Ehrenbergi*, the development of resistant varieties of alfalfa and sweet clover appears to offer the best possibilities. Although the present studies indicate that most of the commonly grown varieties are susceptible to attack by this pathogen, resistant species like *Medicago falcata* may prove valuable as plant breeding material. The possible existence of parasitic races of the fungus is likely to complicate and delay the breeding of resistant varieties. In the meantime, crop rotation is necessary, since *C. Ehrenbergi* increases rapidly in the soil when legume forage crops are grown continuously. Cereal crops are apparently not attacked by the fungus, and it might be advisable to grow them for several years in fields where alfalfa or sweet clover have suffered severely.

Further evidence was obtained in this study that alfalfa and sweet clover are predisposed in the early spring to attack by root-rotting fungi. The critical period for infection usually occurs when the soil is thawing out, and it may last for only a few days. Afterwards, the roots become relatively resistant to invasion by *C. Ehrenbergi*, and, as previously shown by Sanford (23), immune to attack by *Plenodomus Meliloti*. The exact factors concerned in this phenomenon are not yet known. However, since retarded wound-cork formation is apparently not important (9), it seems probable that an altered biological condition of the root tissues, in combination with certain environmental factors, creates the increased susceptibility of the plants as they emerge from the dormant condition.

Acknowledgments

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THE EFFECT OF HIGH TEMPERATURE ON UREDIAL DEVELOPMENT IN CEREAL RUSTS¹

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Abstract

The effect of high temperatures on the development of stem rust and leaf rust on wheat seedlings and stem rust and crown rust on oats seedlings was studied in greenhouse experiments. The experimental results show that, for temperatures above the optimum for rust development, the higher the temperature the less vigorous the pustule development. Physiologic races that at ordinary temperatures produce a "4" type of infection tend to develop a "3" type or an "x" type at higher temperatures. At still higher temperatures the infection type becomes "2" or "1" or even merely necrotic flecks. Physiologic races of the same rust differ in their sensitiveness to temperature. In stem rust of wheat, races that had been inbred by repeated selfings for two or more generations, showed greater sensitiveness to temperature than races collected in the field. Leaf rust of wheat and crown rust of oats were less tolerant of high temperatures than stem rust of wheat.

Introduction

It has been shown in recent years by many investigators of the cereal rusts that temperature has a profound influence on the development of the uredial stage of these rusts on their cereal hosts. From results published by Waterhouse (10), Johnson (4), Gordon (3), and Peturson (8), it is clear that a moderately high temperature (about 75° F.) favors maximum pustule development of physiologic races of *Puccinia graminis Tritici* Erikss. and Henn., *Puccinia graminis Avenae* Erikss. and Henn., and *Puccinia coronata Avenae* Erikss. and Henn., whereas temperatures below 60° F. tend to inhibit pustule development in certain physiologic races on some host varieties.

Melander (6) has shown that the development of *Puccinia graminis Tritici* is almost suppressed at a temperature of 0° to 1° C., although the capacity for normal rust development is generally recovered on exposure of the infected plants to higher temperatures.

Recently, evidence has been secured that excessively high temperatures may result in a somewhat similar inhibition of rust development in cereal rusts. During the extremely hot weather that prevailed at Winnipeg in July, 1936, it was observed that seedlings of Little Club wheat developed resistance to certain physiologic forms of *P. graminis Tritici*, to which this variety is normally susceptible. On June 27 and on July 3, seedlings of Little Club were inoculated with four races of wheat stem rust that normally produce a "4" type of infection. During the period June 27 to July 15 the temperature of the greenhouse rose as high as 116° F., with a mean maximum daily temperature of 99.6° F. and a mean minimum daily temperature of 66.8° F. When notes on rust development were taken on July 15, it was observed that the infection types produced by some of the races were distinctly abnormal.

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Thus Race 9 produced an "x—" type of infection, Race 9 (orange) and Race 36 (Sudan brown) produced infection types varying from necrotic flecks to a "2" type of pustule (Fig. 1). Race 48 (Fig. 1), however, seemed to be uninfluenced by the high temperatures.

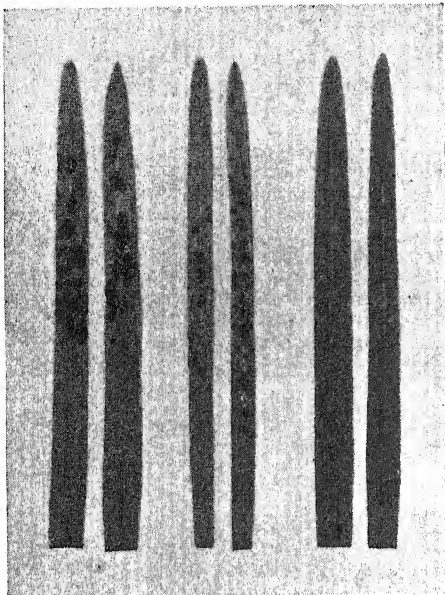


FIG. 1. The effect of high temperatures (mean maximum 99.6° F., mean minimum 66.8° F.) on the infection types produced by two physiologic races of *P. graminis Tritici* on Little Club seedlings. Left, Race 48—unaffected by the high temperature. Right, Race 36 (Sudan Brown)—strongly affected by the high temperature. Centre, Race 36 (Sudan Brown)—at approximately normal temperatures.

With the advent of cooler weather after July 15, observations were made with the object of determining how far the abnormal infections on these plants were capable of recovering their normal rust development. The "x—" type of infection produced by Race 9 soon developed into an approximately normal "4" type of infection, whereas Race 9 (orange) and Race 36 (Sudan brown) showed no further pustule development.

These observations led to a series of greenhouse experiments in which the reactions of normally susceptible varieties of wheat and oats to their respective rusts were studied over a wide range of temperature. The rusts selected for this study were *P. graminis Tritici*, *P. graminis Avenae*, *P. coronata Avenae*, and *Puccinia triticina* Erikss. The range of temperature through which these rusts were studied extended from about 55° F. to over 100° F.

Methods

As the main object of this study was to determine the reactions at various temperatures of varieties which are completely susceptible at ordinary greenhouse temperatures, the experiments were for the most part limited to such varieties as Little Club wheat, which is highly susceptible to most physiologic races of stem rust and leaf rust of wheat, and Victory oats which is susceptible to stem rust and crown rust of oats. Seedlings of these varieties were inoculated and kept overnight in damp chambers maintained at a temperature favorable to infection (about 65°–70° F.). On the following morning the pots containing the seedlings were removed from the damp chambers and distributed in equal numbers among compartments of the greenhouse kept at various relatively constant temperatures, in which they remained until notes were taken on rust development. The experiments were conducted during November and December, 1936, and March and April, 1937.

TABLE I

AVERAGE INFECTION TYPES OF PHYSIOLOGIC RACES OF *Puccinia graminis Tritic* ON LITTLE CLUB WHEAT SEEDLINGS AT TEMPERATURES RANGING FROM 55° TO 99° F.

Race	Source	Mean daily temperature								
		55°-59° F.	60°-64° F.	65°-69° F.	70°-74° F.	75°-79° F.	80°-84° F.	85°-89° F.	90°-94° F.	95°-99° F.
19	Field culture	-	-	4	-	-	-	-	-	3 to 4 c.
21	Field culture	-	-	4	-	-	-	-	-	3 to 4 c.
34	Field culture	-	-	4	-	-	-	-	-	3 to 4 c.
49	Field culture	4	4	-	4	-	-	3+ to x	0	-
56	Field culture	-	-	4	-	-	-	-	-	4-
111	Mars yellow	4-	4-	4	4-	-	0;	-	0	-
21	Barberry (<i>F</i> ₁)	4-	4	-	4	-	-	x+	2+	-
36	Grayish-brown	-	3+	-	4-	3+	-	x-	0;	0;
36	Ochraceous-buff	4-	4-	4	4-	-	1-	-	0	0
52	Grayish-brown	-	4-	4	4	-	-	4-	3 c.	3 c.
57	Orange	-	4-	-	4-	-	-	0;	0	-

* F₁, F₂, F₄ are, respectively, first, third, and fourth generation cultures of crosses between physiologic races. The term "generation", as here used, represents the passage of the rust through its complete life cycle: uredia→telia→aecia→uredia.
c. = sharply chlorotic areas surrounding pustules.

Experiments with Physiologic Races of *Puccinia graminis Tritici*

In view of the fact that the different physiologic races on which observations were made in July, 1936, did not show an identical response to the high temperatures prevailing at that time, it was decided to include in subsequent experiments several physiologic races from various sources. Accordingly these tests included not only races isolated from field collections, but also several races originating in crossing and selfing studies carried out at the Dominion Rust Research Laboratory. In Tables I and II, races from the latter source have been designated by the term "Barberry" to distinguish them from races collected in the field.

Table I shows the infection types produced on Little Club wheat by 11 cultures of wheat stem rust at various temperatures between 55° F. and 99° F. The symbols used to record the types of infection are those originally described by Stakman and Levine (9). The infection types recorded in the table are, in most cases, averages secured from several tests.

An examination of Table I makes it clear that physiologic races differ considerably in their response to high temperatures. It is apparent also that the races collected in the field are less affected by high temperature than those derived from crosses or repeated selfings. The behavior of the field collection of Race 49 at temperatures above 85° F., however, shows that races collected in the field are not all equally resistant to high temperatures. Similarly, there are considerable differences in the temperature responses of the cultures derived from the barberry. Race 52 (grayish-brown), as shown in Table I, and Races 36 and 15 (white), in Table II, exhibit a somewhat greater toleration of high temperatures than other barberry cultures.

TABLE II

THE EFFECT OF TEMPERATURE ON INFECTION TYPE AND NUMBER OF PUSTULES PRODUCED BY FOUR PHYSIOLOGIC RACES OF *P. graminis Tritici* ON LITTLE CLUB SEEDLINGS

Race	Source	Mean daily temperature					
		60.4° F.		78.7° F.		89.7° F.	
		Infection type	Number pustules	Infection type	Number pustules	Infection type	Number pustules
36	Field culture	3+	53	4	10	4	10
36	Barberry (<i>F</i> ₂)	3+	98	4	36	4 c.	6
36	Grayish-brown Barberry (<i>F</i> ₃)	3	79	3+c.	4	x	3
15	White Barberry (<i>F</i> ₄)	3	175	4 c.	33	4 c.	11

Throughout all the experiments, it was noted that the higher the temperature at which the host plants were kept, the fewer pustules developed on the plants. Characteristic results are shown in Table II which records an experiment in which a count was made of the number of pustules formed on the same number of seedlings at three different temperatures. As the infections took place under identical conditions (in this and all other experiments) the sparseness of pustule development at the higher temperatures is clearly the result of a suppression, in an early stage, of mycelial growth in many infections at the higher temperatures.

Experiments with Physiologic Races of *Puccinia triticina*

The results of a number of tests conducted with physiologic races of *Puccinia triticina* are summarized in Table III. The effect of temperature is clearly manifested at temperatures above 85° F. in a tendency on the part of the host plant to develop resistance. It would appear that Race 35 is more sensitive to high temperatures than the other races tested.

TABLE III

AVERAGE INFECTION TYPES OF PHYSIOLOGIC RACES OF *Puccinia triticina* ON LITTLE CLUB WHEAT SEEDLINGS AT TEMPERATURES RANGING FROM 55° TO 94° F.

Race	Mean daily temperature						
	55°-59° F.	60°-64° F.	65°-69° F.	70°-74° F.	75°-79° F.	85°-89° F.	90°-94° F.
5	3+	3+	-	3+	3	x	0;
35	-	4-	-	4-	-	1±	-
76	-	3+	-	-	3	x	-
80	-	3+	3+	4-	-	x-	0; to x-

Experiments with Physiologic Races of *Puccinia coronata Avenae* and *Puccinia graminis Avenae*

The results of these experiments are summarized in Table IV. Seedlings of the variety Victory were used as experimental plants, as this variety is susceptible to races of both rusts. These rusts appeared to be even more

TABLE IV

AVERAGE INFECTION TYPES OF PHYSIOLOGIC RACES OF *Puccinia coronata Avenae* AND *Puccinia graminis Avenae* ON VICTORY OATS SEEDLINGS AT TEMPERATURES RANGING FROM 60° TO 94° F.

Race	Mean daily temperature					
	60°-64° F.	70°-74° F.	75°-79° F.	80°-84° F.	85°-89° F.	90°-94° F.
1 <i>P. coronata Avenae</i>	3+	-	-	-	0 cn.	-
3 <i>P. coronata Avenae</i>	3+	3+	3 c.	3±cn.	3 cn.	-
24 <i>P. coronata Avenae</i>	3+	3+	3+c.	-	3-cn.	1 cn.
6 <i>P. graminis Avenae</i>	4-	4	-	-	-	0 cn.

c.=chlorotic spots. cn.=chlorotic and necrotic spots.

sensitive to temperature than the wheat rusts. At a temperature slightly above 75° F., Races 3 and 24 of *P. coronata Avenae* showed a visible reaction to temperature in the development of sharply chlorotic areas around the pustules. At higher temperatures the chlorosis was largely replaced by necrosis. At temperatures above 85° F. pustules were few, when present at all, but chlorotic spots and necrotic lesions of a brown color were numerous. That the absence of pustules was not due to failure of germ-tube penetration

was demonstrated in leaves of Victory that had been inoculated by *P. coronata Avenae* and kept for 10 days at a mean temperature of 90° F. These leaves, when stained according to McBryde's method (5) of demonstrating rust

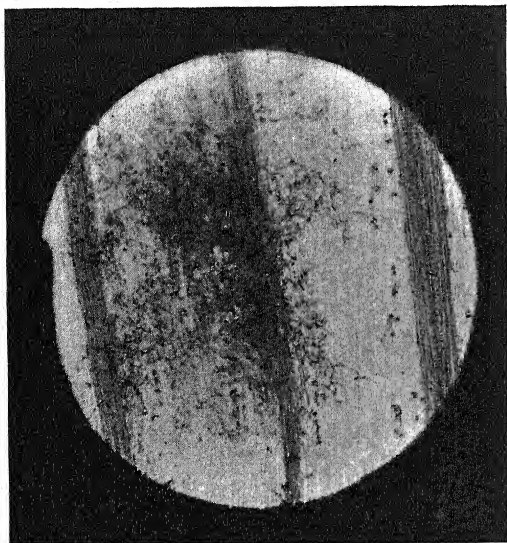


FIG. 2. Mycelium of *P. coronata Avenae*, Race 24, in a 10-days old infection on a seedling of Victory oats kept at a mean temperature of 90° F. $\times 50$.

hyphae in unsectioned leaf tissue, showed here and there mycelia of limited development (Fig. 2). It is not altogether clear what relation, if any, the brown, necrotic areas have to infections. They appeared occasionally on uninoculated, control seedlings at high temperatures but in much smaller numbers than on the inoculated plants. Although no mycelia could be seen in the necrotic lesions when they were stained according to the above-mentioned method, it is possible that they were present but could not be detected owing to the tendency of the dead plant tissue to stain the same color as the mycelium.

The limited experiments on oat stem rust do not permit

any definite conclusions to be drawn but suggest that its behavior towards temperature is similar to that of crown rust.

Discussion

A consideration of the experiments reported in this paper makes it clear that there is an optimum range of temperature for the development of cereal rusts and that progressively higher temperatures militate against normal rust development. Melander (6) has shown that temperatures much lower than the optimum produce a somewhat similar effect. It would appear that the host plants—both wheat and oats—can tolerate a wider range of temperature than their respective rusts. No attempt was made, in the experiments reported above, to determine the absolute upper limit of temperature which wheat or oats will tolerate; and it is, indeed, probable that varieties differ somewhat in this respect. At the highest temperatures tested (mean temp. 97° F.), Little Club wheat did not appear to suffer appreciable injury, providing that soil moisture conditions were satisfactory, whereas even the most vigorous cultures of stem rust tested produced few pustules and these were rather small and surrounded by sharp chlorosis. Leaf rust is apparently less capable of withstanding high temperatures than stem rust; at a mean temperature of 94° F. it failed, for the most part, to produce pustules, and

in one experiment Little Club was rendered highly resistant at a mean temperature of 86° F. Crown rust of oats appears to possess about the same degree of toleration towards temperature as leaf rust of wheat. Experiments on the reaction of stem rust of oats to temperature were too few to permit any definite conclusions.

In stem rust of wheat, and to a lesser extent in leaf rust and crown rust of oats, considerable differences were noted in the sensitiveness of different physiologic races to high temperature. In stem rust of wheat the cultures derived from the barberry were definitely more sensitive to high temperature than those originating in field collections (*Vide* Table I). It must not be inferred from this observation that all cultures derived from aecia on the barberry will behave in a similar way. Most of the "barberry" cultures studied were F_3 or F_4 cultures derived from crosses made several years ago at the Dominion Rust Research Laboratory. The repeated selfings to which such cultures are subjected frequently bring to light various abnormal characteristics such as abnormalities of spore color or a decrease in vigor of sporulation. The appearance of such characteristics is probably due to homozygosity of the factors governing them, a condition brought about by repeated selfings. These factors were undoubtedly present in a heterozygous state in the original rust or F_1 hybrid but, being recessive, they would produce no visible effects. It seems probable that the sensitiveness of the "barberry" cultures to a high temperature is merely one form of degeneration consequent on the continued selfing of physiologic races.

From the point of view of host reaction, the effect of high temperature is expressed in progressively increasing resistance at progressively higher temperatures. The first indication of resistance is the formation of sharply defined chlorotic areas surrounding the pustules. At a higher temperature the infection type ceases to be a "4" or a "3" and becomes an "x" type, that is, pustules of a resistant and susceptible type are intermingled. At still higher temperatures the infection types become "2" or "1" or even merely necrotic flecks. Thus a variety, susceptible at moderate greenhouse temperatures, may exhibit various degrees of resistance at higher temperatures.

The question of whether the host plant is able to maintain a resistance thus acquired, if transferred to a lower temperature, was not thoroughly investigated. A few experiments were performed in an attempt to gain some information on this point. Seedlings of Little Club, which had acquired resistance to stem rust at a high temperature, were subsequently kept at a moderate temperature to determine to what extent the usual infection type of the rust was recovered. The results were not entirely consistent. On certain leaves, which bore only necrotic or chlorotic flecks at the high temperature, pustules of a "3" or "4" type would later develop at the lower temperature. On other leaves, the resistance acquired at the high temperature would be retained at the lower. The mycelium, therefore, had survived in some infections but not in others. A sufficiently long exposure of the mycelium to high temperatures would probably lead to its destruction in all of the infections.

It is probable that the response of these rusts to high temperature has some significance in their epidemiology. That such is the case for *Puccinia glumarum* has been established by Gassner and Straib (1, 2) who have used the term "Sommerresistenz" to designate the resistance which many varieties develop towards stripe rust in the summer months. The sensitiveness of *P. glumarum* to high temperature has also been invoked by Newton and Johnson (7), to account for the failure of stripe rust to spread in the prairie provinces of Western Canada during the midsummer period. It is possible that the relatively smaller damage done by the leaf rust of wheat and crown rust of oats in the great plains region than by stem rust is to some extent attributable to a similar cause. The behavior of leaf rust of wheat particularly is suggestive. In Manitoba this rust usually appears early in the summer and spreads considerably while the weather is still cool. With the advent of warmer weather, leaf rust makes much slower progress than stem rust although the latter appears somewhat later in the season. Possibly the greater resistance of stem rust to high temperature may, at least in part, account for its rapid development in periods of warm weather.

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STIMULATION OF CAMBIAL ACTIVITY, LOCALLY IN THE REGION OF APPLICATION AND AT A DISTANCE IN RELATION TO A WOUND, BY MEANS OF HETEROAUXIN¹

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Abstract

The application of heteroauxin in lanoline (1 mg. of heteroauxin per gm. of lanoline) to the distal end of disbudded cuttings of leader shoots of balsam poplar, stimulated cambial activity for a distance of 1.0–1.5 in. below the point of application. Marked stimulation of local cambial activity, in relation to a bridged ring some considerable distance below the point of application of the heteroauxin, was also obtained. The response at the wound was distinct and separate from the response in the region of application of the heteroauxin, since in the intervening distance no cambial activity had occurred. The experiments were carried out during the winter months, so that the cambium was dormant in material as it came from the field. Cambial activity subsequent to treatment was estimated in terms of xylem formation. The structural features of this new xylem are described and discussed, with particular reference to the question as to whether heteroauxin stimulates cell division only in the cambium or, in addition, is active in differentiation of typical xylem elements.

Introduction

In a recent contribution Brown (2), using leader shoots of balsam poplar, showed that in disbudded cuttings the greater the amount of living bark distal to a bridged wound, the greater is the development of local cambial activity in relation to the wound. It was also shown that local wound-cambial activity is stimulated further by the presence of developing buds and leaves distal to the wound. This work was done during the winter months, at a time when the cambium was dormant in material outdoors, which rendered it easy to measure cambial activity subsequent to treatment. On the basis of the quantitative results obtained, it was concluded that a hormone, present in the living bark and produced also by developing extension growth, is involved in local wound-cambial activity. Moreover, it was argued further that this hormone is probably identical with that which emanates from developing extension growth to promote the basipetal development of normal cambial activity.

The concept of hormone regulation of normal cambial activity receives strong support from the fundamental investigations of Avery, Burkholder and Creighton (1), and of Söding (6), who have definitely established a close parallelism between the intensity of cambial activity and growth-hormone concentration. In their experiments, the concentration of growth hormone was estimated in terms of the *Avena* coleoptile test, and it is implied that the hormone promoting cell extension or elongation promotes cell division in the cambium also. Such a conclusion had been reached at an earlier date by Snow (4), who suggested that the cambial hormone and the growth hormone were identical, and might indeed be auxin-*a*.

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Snow (4) succeeded in activating the cambium in decapitated stems and hypocotyls of sunflower seedlings by applying to their upper ends weak solutions in gelatine of pure heteroauxin (3-indole acetic acid) and of auxin-*a*. Söding (5) obtained activation of the cambium in shoots of various trees as a result of application of pure heteroauxin. The twigs were ringed towards the end of March, and buds below the ring were removed. About two months later, he made a longitudinal incision running down 2-3 cm. from the lower margin of the ring, a flap of bark was lifted up and crystalline heteroauxin applied directly to the exposed cambium. After periods of 16-21 days and one month, in the experiments of Snow and Söding respectively, stimulation of the cambium was observed for a few centimetres below the point of application of the auxin. According to Söding (6) similar results with heteroauxin have recently been obtained by at least two other European investigators.

Now although heteroauxin, unlike auxins *a* and *b*, has never been isolated from the higher plants, experiments with this substance upon higher plants are of interest, because in all the phenomena which have so far been investigated, it has acted in the same way as the other auxins. With regard to wound-cambial activity, it is clearly of interest to determine whether it is possible to stimulate cambial activity in the vicinity of a wound by the application of heteroauxin at a point some distance above the wound, and in such a way that any response at the wound is distinct from any development arising in the region of application of the auxin. Such a result would be analogous to that obtained (2) when developing buds and leaves are present distally on a cutting, since stimulation of local cambial activity in relation to a wound below can be observed definitely before the basipetal gradient of normal cambial activity, emanating from the developing extension growth, has reached the level of the wound.

The difficulty in explaining local cambial activity in relation to bridged wounds, wholly in terms of the action of a single hormone, has already been discussed by Brown (2), who has suggested a hypothesis involving interaction between the hormone and a definite wound substance. This however is another problem and the original paper should be consulted for details.

Experiments and Results

Leader shoots of *Populus balsamifera* L., the balsam poplar, were used throughout this investigation. In the first experiment to be described, the three-year-old portions of six leader shoots were selected (December 29, 1936), and these lengths of shoot were completely disbudded by the excision of all lateral branches. Each length of shoot was then cut to form a set of three units of equal length, which will be designated as the upper, middle and lower units in each set. Although the three units in any one set were all of equal length, the different sets varied in length from 12 to 15 inches.

In each set a similar longitudinally bridged ring was made at the same distance from the morphologically upper end in all three units. The distance

from the upper end of the unit to the bridged ring below varied from 7–10 in. in different sets. Likewise the length and width of the longitudinal bridge of bark varied somewhat in different sets, the average length of the bridge being about $\frac{3}{4}$ in., and the width approximately $\frac{1}{12}$ of the circumference of the unit. The distance between the bridged wound and the basal end of the unit was from 4 to 5 inches.

Variation between different sets is of no import in so far as this work is concerned, and is indeed difficult to avoid in many cases, simply because of the constitution of the material with respect to variation in thickness of the shoots, in the length of internodes, and so on. What is important is to ensure, as far as is possible, uniformity between units within a set, and this was carefully attended to in these experiments. The bridged wound was smeared with vaseline and the units set up in a vertical position with their basal end in about one inch of tap water. It is necessary to keep all the units vertical since, as Brown (2) has shown, gravity has a marked influence upon cambial activity in relation to wounds.

About two weeks later (January 13, 1937) some of the bark was scraped off all around for a short distance ($\frac{1}{2}$ in.) from the upper end of all units, to expose the living phloem beneath. In two units of each set, namely the upper and lower, the exposed tissue was smeared liberally with a lanoline paste (1 part anhydrous woolfat to 1 part distilled water) containing 1 mg. of pure heteroauxin per gm. of lanoline. The heteroauxin (3-indole acetic acid) was a sample purchased from Merck and Co. The middle unit served as a control and was treated similarly, except for the fact that the lanoline paste contained no heteroauxin. On January 29, and again on February 3, the respective lanoline pastes were removed and renewed, and on each occasion the previously exposed phloem was scraped again to present a fresh surface. The experiment terminated four weeks after the first of the three treatments with heteroauxin (February 2, 1937), when the bark was peeled off entirely and the units allowed to dry out.

On drying out, any new xylem laid down by the cambium during the experiment shows up clearly upon the surface of the old wood. Fig. 1 is a photograph of a typical set showing the response at the bridged wound, and at the distal end of the upper (*a*), middle (*b*) and lower (*c*) units. The material illustrated was first photographed without any previous treatment, except for the fact that the new xylem was outlined with India ink. However, it became clearly evident that there was insufficient differentiation in the photograph to allow for reproduction, and accordingly the following procedure was resorted to. Powdered brown chalk was rubbed lightly into the wood with the finger. The chalk adhered readily to the rough surface of the new xylem but not to the smooth surface of the old, and in this way marked differentiation was obtained. It will be observed in the upper and lower units, which were treated with heteroauxin, that a basipetal gradient of new xylem has been laid down for a short distance (1.0–1.5 in.) from the distal end of the units, whereas in the middle control unit no such response is evident.

But of still greater interest is the fact that there is obviously much more new xylem in the vicinity of the bridged wound below in the treated units *a* and *c*, relative to that in the control unit *b*. Moreover, the response at the bridged wound in the treated units is distinct and separate from the response at the distal end, since no new xylem had been laid down in the intervening distance, except locally in some cases at the base of excised lateral shoots. Transverse

TABLE I

NUMBER OF VESSELS IN A TRANSVERSE SECTION THROUGH THE LONGITUDINAL BRIDGE OF UNITS TREATED WITH HETEROAUXIN AND OF CONTROL UNITS

Upper (treated)	Middle (control)	Lower (treated)
330	80	290
286	53	333
351	84	344
330	33	460
167	15	201
106	17	133

sections were made through the middle of the longitudinal bridge in all units, and the number of vessels counted. Table I shows the results obtained for the six sets. The first set in Table I is that which had been photographed to provide Fig. 1. These vessel counts indicate clearly that the application of a heteroauxin paste some distance above has greatly stimulated the cambium in the vicinity of a bridged ring below. In every case, the response at the bridged ring was distinct and separate from the response in the region of application of the heteroauxin.

Similar results were obtained in another experiment. In this case the four-year-old portions of twelve leader shoots were selected (December 29, 1936). These portions were completely disbudded and each portion then cut to form a set of two units of equal length, which will be designated as the upper and lower units of each set. In each set a longitudinally bridged ring was made in both units at the same distance from the morphologically upper end. The average length of the units was 15 in., the average distance between the morphologically upper end and the bridged wound below, 9 in., the average length of the longitudinal bridge of bark $\frac{3}{4}$ in., the average width of the bridge $\frac{1}{2}$ of the circumference of the unit, and the average length of shoot below the bridged ring was $5\frac{1}{4}$ in. Just as before, these lengths varied between sets but were the same for the two units within any one set. The bridged wound was vaselined and the units set up vertically with their basal end in tap water.

The material was treated immediately (December 29, 1936) with heteroauxin. Some of the bark was scraped off all around at the upper end for a distance of about $\frac{1}{2}$ in. to expose the living phloem beneath. In six sets a lanoline paste, containing one mg. of heteroauxin per gm. of lanoline, was applied liberally to the exposed phloem at the end of the lower units. The six upper units served as controls and were treated with lanoline only. In the other six sets the upper units were treated with heteroauxin and the lower units served as controls. The respective pastes were removed and renewed at weekly intervals, at which times the phloem was scraped again to expose a fresh surface. The experiment terminated (February 3, 1937) five weeks after the first of five treatments. As in the previous experiment, the bark was peeled off completely and the material allowed to dry out.

The results were precisely the same as before. In the units treated with heteroauxin, a basipetal gradient of new xylem had been laid down for a distance of 1.0–1.5 in. from the upper end, whereas no such response was obtained in the controls. There was a marked increase in the amount of new xylem in the vicinity of the bridged wound in treated units relative to the controls, and again there was no evidence of cambial activity in the region between the bridged ring and the basipetal gradient of new xylem just below the point of application of the heteroauxin. A quantitative estimate of the difference between the extent of cambial activity at the bridged ring, in treated and control units, is presented in Table II, in which is set forth the number of vessels in transverse sections through the middle of the longitudinal bridge of all units.

Examination of the new xylem, laid down in a basipetal gradient for a short distance just below the region of application of the heteroauxin, showed it to be abnormal in some respects. The wood was characterized by abundant nests of parenchyma, and the vessels were rather narrow on the whole. These abnormalities were most marked at the extreme end of the unit, and the wood became more and more typical at increasing distances from the cut end. Exactly similar observations have been reported by Söding (5) for balsam poplar and a number of other trees. Fig. 2 is a photograph of a transverse section cut at about $\frac{1}{2}$ in. from the distal end of a unit treated with heteroauxin. The section was stained with phloroglucin, a lignin stain, and shows clearly the unstained nests of parenchyma in the new xylem.

Now although there was no obvious response below the distal end of control units, it would not be correct to say that the cambium had remained entirely inactive. Brown (2) has already shown that a certain amount of cambial activity does develop from the lower margin of a complete ring, or what is really the same thing, from the distal cut end of a cutting. Under such conditions a basipetal gradient of cambial activity, very much feebler and less extensive than that obtained when heteroauxin is present, can be observed. The cambium cuts off cells which remain more or less uniformly rectangular in shape, and thin-walled. Vessels and fibres are not differentiated, although a few tracheids may be formed. For a fuller discussion and illustrations of this type of development, the earlier paper (2) should be consulted. It is quite clear, however, from these present experiments, that the effect of heteroauxin is not simply to induce some degree of differentiation in layers of cells which would have been formed, as in the controls, in the absence of hetero-

TABLE II
NUMBER OF VESSELS IN A
TRANSVERSE SECTION
THROUGH THE LONGITUDINAL
BRIDGE OF UNITS TREATED
WITH HETEROAUXIN AND OF
CONTROL UNITS

Upper (control)	Lower (treated)
95	335
56	306
57	381
110	405
60	343
76	282
Upper (treated)	Lower (control)
503	136
313	114
392	77
118	54
243	85
259	78

auxin. Heteroauxin definitely stimulates cell division for a short distance below the point of application.

When the new wood laid down locally in relation to the bridged wound was examined in treated and control units, the following differences, apart from the obvious difference in amount, were noted. Lignification of the new xylem was more marked and there was a tendency for the vessels to be wider in the units treated with heteroauxin than in the controls. It is of interest that similar differences have already been observed by Brown (2) between units bearing developing extension growth, and completely disbudded units. In addition, nests of parenchyma were less common in the new wood formed locally, in relation to the bridged ring, in units treated with heteroauxin than in the controls.

In every unit treated with heteroauxin there was less parenchyma in the wood laid down in the longitudinal bridge of the ring below, than in the basipetal gradient of new xylem in the region of application of the heteroauxin. The wood in the longitudinal bridge in treated units was, generally speaking, quite normal in appearance. Lignification was more marked, and invariably the vessels in this region were wider than the vessels in the basipetal gradient at the distal end of the same unit. Some of these differences are illustrated in Figs. 2, 3 and 4. The material all belonged to the set appearing first in Table II. Fig. 2 is a transverse section about $\frac{1}{2}$ in. below the distal end of the unit treated with heteroauxin; Fig. 3 is a transverse section through the middle of the longitudinal bridge of the same unit; and Fig. 4 is a transverse section through the middle of the longitudinal bridge of the control unit. Only small portions of the bridge are shown in Figs. 3 and 4, but they are from corresponding positions in both units. It will be observed that the wood in the longitudinal bridge of the control unit rather resembles that formed just below the region of application of the heteroauxin in the treated unit. All the sections were stained with phloroglucin in the presence of hydrochloric acid.

The question of tissue orientations in relation to bridged wounds has already been discussed by Brown (2), and nothing would be gained by reconsidering it at this time. Suffice it to say that the tissue orientations round the longitudinally bridged wound were exactly the same in units treated with heteroauxin and in controls.

In all units, a basifugal gradient of xylem was laid down over a short distance at the basal end. This type of development is well known, having been first observed by Hartig (3) in 1862, and commented upon by several investigators since that time. In the present experiments, this basifugal gradient was always more extensive in units treated above with heteroauxin than in the control units, which is just what might be expected. The interesting point, however, is that the new xylem was always markedly "piled up" in the control units, causing a definite bulge just above the cut end, whereas in the treated units the same degree of "piling up" was never evident. In other words, the basifugal gradient of new xylem in the controls was short and steep, whereas

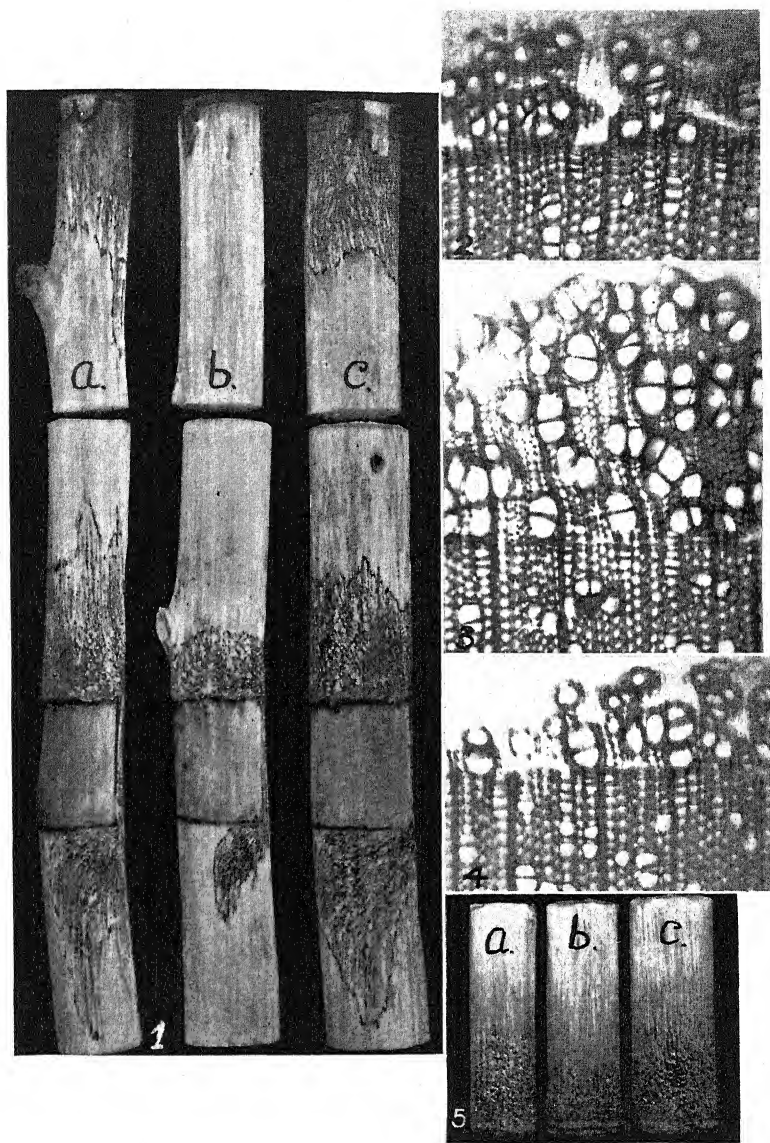


FIG. 1. Cambial activity at the distal end and around a longitudinally bridged ring below, in three units derived from the three-year-old portion of one leader shoot of balsam poplar. The upper (a) and lower (c) units were treated at the distal end with heteroauxin in lanoline. The middle unit (b) served as a control and was treated with lanoline only. $\frac{5}{8}$ nat. size.

FIG. 2. Transverse section, cut $\frac{1}{2}$ -inch from the distal end of a unit treated with heteroauxin. $\times 85$.

FIG. 3. Transverse section through the middle of the longitudinal bridge of the same unit as in Fig. 2. $\times 85$.

FIG. 4. Transverse section through the middle of the longitudinal bridge of the control unit corresponding to that in Figs. 2 and 3. The sections in Figs. 2, 3 and 4 were stained with phloroglucin and hydrochloric acid. Only a small portion of the longitudinal bridge is shown in Figs. 3 and 4, but from corresponding positions in both cases. $\times 85$.

FIG. 5. Cambial activity at the basal ends of a set of three units, derived from the three-year-old portion of one leader shoot. The upper (a) and lower (c) units were treated at the distal end with heteroauxin and the middle unit (b) served as a control. $\frac{5}{8}$ nat. size.

in the treated units the gradient, although much longer, was much less steep relative to the controls. Indeed, it was quite common to find a greater thickness of new xylem in the region of "piling up" in the control units, than at any point in the basifugal gradient of the corresponding units treated with heteroauxin. Some of these points are illustrated in Fig. 5, which is a photograph of the basal end of the upper (a), the middle (b) and the lower (c) units of the set appearing second in Table I. The material was treated with powdered chalk as previously described.

These observations with respect to the basifugal gradient of xylem at the basal end of units are incidental, to the extent that they do not effect the main conclusions to be derived from these experiments. They may, however, prove to be of significance in other connections, and since they were a constant feature of the experiments, the following tentative explanation is submitted for consideration. If, in the control units, there is a mass movement downwards of growth hormone present in the living bark, there would be a tendency for this hormone to accumulate at the basal end to give rise to a concentration gradient, highest at the base and decreasing in the upward direction. It is also probable that polarity within the unit would tend to steepen this gradient, whereby it is conceivable that the concentration of growth hormone would become, and would be maintained, sufficiently high to stimulate cambial activity, only over a very short distance at the basal end. In which case, the growth hormone would be effective within a very short distance, thus accounting for the "piling up" effect in control units. In the units treated with heteroauxin, there would again be a tendency to form a similar concentration gradient of growth hormone at the basal end as a result of mass movement downwards. In addition, however, there is a concentration gradient of heteroauxin in the opposite direction; *i.e.*, decreasing from the source above downwards. It is clear that on reaching the basal end, the effect of the heteroauxin would be to lengthen the effective concentration gradient of cambial stimulant (in this case growth hormone plus heteroauxin), and in addition to decrease the steepness of that gradient. In this case, the concentration of heteroauxin and growth hormone at the basal end would become sufficiently high to stimulate cambial activity over a considerably greater distance, and without the same degree of "piling up" relative to that obtaining in the controls.

Discussion

The foregoing experiments confirm the earlier conclusions of Snow (4), Söding (5) and others, to the effect that heteroauxin stimulates cambial activity in the shoots of plants for a short distance below the point of its application. Heteroauxin applied at the distal end of a shoot cutting will also stimulate cambial activity around a bridged wound below. Of particular interest is the fact that the response at the wound below is distinct and separate from the response in the region of application of the heteroauxin. The simplest explanation is that the heteroauxin travels down a considerable length of shoot without stimulating the cambium in its path, but does,

however, on reaching a bridged ring below, stimulate markedly local cambial activity at that point. It is quite probable that the bridged wound presents an obstacle to the downward movement of the heteroauxin which will, in consequence, tend to accumulate just above the wound. There is a clear analogy here with the type of behavior obtained when developing extension growth is present distally on a cutting. Brown (2) has shown that local cambial activity in relation to a bridged ring is stimulated by the presence of developing buds and leaves distal to the ring, and that this response can be observed before the normal basipetal development of cambial activity, emanating from the extension growth, has reached the wound. In this connection it was suggested that the hormone emanating from the extension growth must move to some extent in advance of the basipetal development of cambial activity. This suggestion has since been proved correct by Avery, Burkholder and Creighton (1), who have shown definitely that growth hormone moves basipetally in stems in advance of cell division in the cambium. Likewise, Söding (6) found by analysis of plant parts that growth hormone appears first and is followed later by cambial activity.

Söding (5, 6) has recently developed the hypothesis that cambial activity can of itself produce growth hormone. He considers the production of growth hormone in expanding buds to be prerequisite to the initiation of cambial activity in the shoot immediately below, where much of the growth hormone is used up. He then suggests that the activated cambium produces further supplies of growth hormone, which travel but a short distance downwards to repeat the process. In terms of this hypothesis, according to Söding, a molecule of growth hormone some distance down a tree has not travelled to that point from far above, but was manufactured, either in the place in which it finds itself or but slightly above. However, Brown (2) has attributed the stimulation of local wound-cambial activity, when developing extension growth is present on a cutting, to movement of growth hormone in advance of the basipetal development of normal cambial activity. It is admitted that the distance between the advancing front of the basipetal development of cambial activity emanating from the extension growth and the bridged wound below, was never more than a very few inches at the time the degree of stimulation at the bridged ring was estimated. Movement of growth hormone over the space of a few inches might well fall within the terms of Söding's hypothesis. On the other hand, it is quite probable that stimulation of cambial activity at the bridged wound could have been detected earlier, although less clearly of course, at times when greater distances intervened between the wound and the advancing front of cambial activity from above. Under these circumstances, movement of growth hormone over correspondingly greater distances would have to be admitted. The foregoing experiments with heteroauxin are, by analogy, of considerable interest in this connection. Distances as great as nine inches intervened between the lower limits of the basipetal development of cambial activity in the region of application of the heteroauxin and the bridged wound below, where marked stimula-

tion of cambial activity was observed. Still greater distances intervened between the region of application of the heteroauxin and the basal end of cuttings, where the effect of the heteroauxin was also manifested. It would appear reasonable to suppose that the heteroauxin had travelled over these distances, and there is no reason to believe that still greater distances could not be traversed.

Söding (5, 6) has expressed the opinion that growth hormone stimulates cell division only in the cambium, and that other factors are necessary for the differentiation of typical xylem and phloem. This opinion appears to be based mainly on the results of his experiments with heteroauxin, where he found that the new xylem formed just below the point of application of the heteroauxin was abnormal. Differentiation was incomplete in so far as an abundance of parenchyma was present, and to the extent that the vessels were often quite narrow. Similar results were obtained in the present experiments with respect to the xylem formed in the region of application of the heteroauxin, although as Söding (5) also observed, the xylem became more typical at increasing distances below the point of application of the heteroauxin. Söding (6) has refuted the suggestion that an inhibiting effect, due to high concentrations of heteroauxin at the point of application, is the sole cause of incomplete differentiation, since he obtained similar results with weak concentrations. He adheres to his previous suggestion that heteroauxin stimulates cell division only and that other factors are necessary for the differentiation of typical xylem and phloem. However, in view of the results of the present investigation, it might be argued that heteroauxin does indeed stimulate both cell division and differentiation, as is apparently the case in the longitudinal bridge of treated units, and that some other factor limits differentiation at the distal end. It should be recalled that the degree of differentiation at the distal end increases at increasing distances from the region of application of the heteroauxin, which would indicate the action of some purely local factor at more distal points.

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CHEMICAL WEED KILLERS

III. RELATIVE TOXICITY OF SEVERAL CHEMICALS TO PERENNIALS UNDER FIELD CONDITIONS¹

By W. H. COOK², T. K. PAVLYCHENKO³, J. M. MANSON², AND P. GARROW⁴

Abstract

Of 15 chemicals applied to perennial weeds over the same range of dosages, only five appear to possess a useful toxicity as judged by the number of living plants 12 months after treatment. The effective chemicals can be classified into three groups according to their toxicity, (i) sodium chlorate; (ii) barium chlorate and arsenic pentoxide; and (iii) ammonium thiocyanate and sodium arsenite. The relative toxicity of these three groups of chemicals, judged from the certainly lethal dosage, appears to fall in the proportions of 1 : 1.5 : >2.

Introduction

The relative toxicity of several chemicals to perennial weeds under field conditions was determined in this study. Of the chemicals used, 13 were selected from the 19 substances found to be most toxic to annual weeds in an earlier investigation (2). Two substances less toxic to annual weeds, barium chlorate and α -naphthylamine, were also included, the first because chlorates are generally effective, and the barium salt is less of a fire hazard than the sodium salt (1); and the second because it appears to have a residual toxic effect in the soil (2), a property which may be necessary for the eradication of perennial weeds.

Two series of tests were made: one near Edmonton in 1932 included tests of 11 chemicals on two species, and the other at Saskatoon in 1933 included tests of seven substances on one perennial weed. In the last series several of the earlier tests were repeated so that only 15 different substances were used. These two sets of experiments are subsequently designated the "Edmonton" and "Saskatoon" series respectively.

Materials and Methods

EDMONTON SERIES

The following chemicals were used: sodium hydroxide, sodium arsenite, sodium chlorate, barium chlorate, ammonium thiocyanate, sodium cyanide, zinc chloride, sodium dichromate, phenol, creosote and tar acids, *i.e.*, the acid sludge from oil refineries. Soluble substances were applied as a 10% solution with a hand sprayer, and the creosote and tar acids were used as a 10% suspension and sprinkled on the plants with a watering can. Three dosages of each substance were applied, namely, 700, 750 and 800 lb. of active constituent per acre.

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One set of plots was laid out in a strong stand of couch grass *Agropyron repens* (L.) Beauv. with a few Canada thistles *Cirsium arvense* (L.) Scop., and annual weeds. Adjacent areas 12 ft. square were staked out before treatment. Later, pathways 4 ft. wide were cut out and subsequent observations made on the remaining 100 sq. ft. This procedure excluded the interfering marginal effects resulting from overlapping treatments, or accidentally untreated areas. Each dosage was applied to three plots, and a single plot was treated with each chemical at a rate of 750 lb. per acre, after mowing the weeds.

A less extensive series of treatments was made in the same manner in an area supporting a moderately strong stand of Canada thistle. This series included all the chemicals and dosages listed above, but the greatest and smallest doses were applied to single plots, while the 750 lb. per acre treatment was made in duplicate. Treatments were applied during bright, warm weather in early July. A few evening showers occurred during the period but chemicals were invariably applied to dry vegetation.

SASKATOON SERIES

These tests included the following chemicals: arsenic pentoxide, sodium chlorate, creosote, tetralin, aniline, α -naphthylamine and tar acids. The first two chemicals are soluble in water and were applied as a 10% solution. As the others are either insoluble, or not sufficiently soluble in water to make a 10% solution, some laboratory studies were made on the emulsification of these materials prior to making the tests. This was felt to be necessary since the results of the Edmonton tests indicated that creosote and tar acids were relatively ineffective, probably because they were applied in rather coarse suspension. These laboratory experiments indicated that a suitable emulsion of creosote could be prepared in 0.2% sodium hydroxide; tetralin and aniline could be emulsified, soap or glue being used as the emulsifying agent, while α -naphthylamine could be dissolved in a small quantity of a solvent (tetralin was used) and emulsified with glue as a stabilizer. The small quantities of these emulsions used in the laboratory tests could be prepared without extremely vigorous agitation, and applied through a spray nozzle. In large containers, however, the emulsions separated into layers of different concentration, and vigorous agitation was necessary before transferring to a sprayer. Most of the emulsions also plugged the spray nozzles rather easily, and to avoid this difficulty a sprinkling can was finally used to make all applications. All chemicals were again applied at three dosages, but as the three rates of application used in the Edmonton series gave essentially similar results, the dosage intervals in these tests were increased, the applications being 600, 750 and 900 lb. per acre of the active constituent.

Plots of the same size as those in the Edmonton series were laid out in a field infested with a vigorous growth of Canada thistle. All dosages were applied to triplicate plots. The treatments were made in the latter part of July, during warm, bright weather, with winds of moderate velocity.

Results

EDMONTON SERIES

The results obtained from the couch grass plots appear in Table I. Three distinct kinds of observations and data were obtained: first, the effect of the chemical on the original herbage; second, the rate of development and character of new plants, and finally, the number of plants per unit area the year following the treatment. The last measurement gives the best estimate of the efficacy of the treatment, because a significant reduction in the number of plants in a treated area, as compared with an untreated one, gives some idea of the true mortality. On the other hand, records taken during the year of treatment give some idea of the rate of killing and the extent of re-growth; but since certain substances can kill the aerial parts

TABLE I
TOXICITY OF CHEMICALS TO COUCH GRASS
(Edmonton series)

Chemical	1932 records		1933 record
	Effect on original plants	Effect on development of new plants	No. plants per sq. yd. as % of untreated plots
Sodium chlorate	Slow killing, all dead at freeze-up	Few weak discolored plants	3
Barium chlorate	Slow killing, few stems alive at freeze-up	More new growth than in NH_4CNS or NaClO_3 plots	12
Ammonium thiocyanate	Toxic action evident immediately, followed by slow bleaching	Practically no new growth	30
Sodium arsenite	All original herbage killed	Vigorous new growth developed immediately	48
Sodium cyanide	Bleached within two days, apparently completely killed	Considerable new growth	125
Tar acids* (acid sludge)	Almost complete kill	Tall growth of new plants	150
Sodium dichromate	Immediate discoloration and rapid killing	Thick, vigorous new growth three weeks after treatment	155
Phenol	Slow killing, many original stems alive at freeze-up	New growth, rather sickly and thin	170
Sodium hydroxide	Affected plants immediately, most of original growth severely damaged	New growth appeared early and developed luxuriantly	175
Creosote*	Immediate wilting but plants made good recovery, only partial kill resulted	—	187
Zinc chloride	Slow killing of most of original herbage	New growth of rather tall weak plants	246

*Difficult to apply satisfactorily.

of the plant without seriously affecting the roots, it is impossible to estimate the permanent effect from such observations. It was found that whenever a chemical failed to kill the original herbage completely there appeared to be less tendency for new growth to appear, but owing to the large number of living plants in evidence under these conditions it is difficult to establish this point.

In no case was there any detectable difference between the different rates of application or between the block of mowed and unmowed plots. Observations and counts were made in all plots but these are grouped or averaged for all doses of the same material in Table I. The weed counts made in 1933 were taken at approximately three-week intervals in the months of May and

TABLE II
TOXICITY OF CHEMICALS TO CANADA THISTLE
(Edmonton series)

Chemical	1932 records		1933 record
	Effect on original plants	Effect on development of new plants	No. plants per sq. yd. as % of untreated plots
Sodium chlorate	Slow killing, all dead by freeze-up	Few weak, sickly plants	0
Barium chlorate	Slow killing, all dead by freeze-up	Few weak, sickly plants	0
Ammonium thiocyanate	Slow killing, all dead by freeze-up	Few weak, sickly plants	50
Sodium arsenite	Original herbage apparently partly killed, some recovery	Considerable new growth	120
Sodium cyanide	Original herbage killed	Profuse new growth	170
Tar acids* (acid sludge)	Only partial destruction and good recovery	New growth very uneven, apparently normal plants interspersed with few small sickly ones	110
Sodium dichromate	Complete destruction	Plentiful new growth appeared shortly after treatment, some of these plants died subsequently	110
Phenol	Complete destruction	Plentiful growth appeared late in season	120
Sodium hydroxide	Rapid and complete destruction	Plentiful growth, some showing signs of weakness	82
Creosote*	Injured upper portion of plant only, recovery from unaffected growth below	Plentiful new growth appeared normal	120
Zinc chloride	Rapid killing but probably incomplete destruction	Luxuriant growth of new plants	110

*Difficult to apply satisfactorily.

June. These usually increased, in the plots unaffected by treatment, as the season advanced, but they were again averaged in reducing the data. Since the plants were counted three times on four square-yard areas, chosen at random in each plot, and as ten plots were treated with each chemical, the average figure presented in the last column of Table I is the result of 120 counts. Statistical analysis showed that the first three chemicals reduced the number of weeds significantly as compared with the untreated check, while the fourth, sodium arsenite, was just on the border line of significance. All the other treatments increased the number of plants as compared with the untreated plots, but most of these differences are not statistically significant.

The results of similar treatments on Canada thistle appear in Table II, the chemicals being arranged in the same order as in Table I. Here also there was no detectable difference between the three rates of application, and the results on all plots were again grouped and averaged. In 1933 the number of plants per square yard was counted as before, three times at three-week intervals, but as there were only four plots of this weed treated with each chemical, the numbers in the last column are the average of only 48 counts. The two chlorate salts killed all the plants, and statistical analysis showed that ammonium thiocyanate reduced the number of weeds significantly. With the exception of sodium cyanide, where stimulation was evident, none of the other treatments differed significantly from the controls.

SASKATOON SERIES

The results obtained in the Saskatoon experiments appear in Table III, the figures representing the average results from triplicate plots. More detailed records were taken during the year of treatment, the number of dead leaves, stems, and new plants being counted one day, one week and one month after treatment, followed by a final observation made just before freeze-up. These results obtained at the different rates of application appear separately, as the larger intervals between doses gave significantly different results in some instances.

It can be seen from Table III that the majority of the chemicals killed most of the leaves and stems on the original plants. No new plants were evident in any of the plots one week after treatment, but subsequent observations showed considerable growth, except in the plots treated with sodium chlorate and arsenic pentoxide, where only a few plants developed.

In 1934 detailed records were again taken on all plots, but as sodium chlorate and arsenic pentoxide were the only effective chemicals, the results are merely reported in a descriptive form in Table III. A dosage of 600 lb. per acre appears to be about the certainly lethal dose of sodium chlorate to Canada thistle under the conditions of this experiment, while about 900 lb. of arsenic pentoxide is required to produce complete mortality. It is likely that smaller dosages of these chemicals would effect a useful, though incomplete, mortality.

TABLE III
TOXICITY OF CHEMICALS TO CANADA THISTLE
(Saskatoon series)

Chemical	Dosage, lb. per acre	Average number plants per sq. yd.	1933 records—Dead leaves and stems and number of new shoots per sq. yd. at intervals after treatment										1934 records
			One day		One week		One month			Before freeze-up			
			Dead leaves, %	Dead stems, %	Dead leaves, %	Dead stems, %	Dead leaves, %	Dead stems, %	New shoots, No. per sq. yd.	Dead leaves, %	Dead stems, %	New shoots, No. per sq. yd.	
Sodium chlorate	600	30.6	80	16.6	100	55	100	96.6	2.6	99.6	98.3	1	No growth
	750	31	95	25	100	80	100	98.3	1	100	100	0	No growth
	900	32.6	100	28.3	100	91.6	100	100	0	100	100	0	No growth
Arsenic pentoxide	600	30.3	100	28.3	100	96.6	100	100	0	100	100	0	A few sickly looking thistles. Many wild barley seedlings
	750	30.3	100	40	100	98.3	100	100	0	100	100	0	No thistle but some wild barley
	900	30.3	100	38.3	100	100	100	100	0	100	100	0	No growth
Acid sludge	600	26.6	100	85.5	100	100	100	100	38	100	100	44.3	Thick and luxuriant growth
	750	31.3	100	88.3	100	100	100	100	47	100	100	59.3	
	900	32	100	96.3	100	100	100	100	46	100	100	57.3	
Alpha-naphthylamine	600	26	73	0	100	31.6	100	83	15.6	100	96.6	31	Thick healthy growth of the weed and grasses
	750	26	83.6	0	100	43.3	100	96.6	30.3	100	100	43.6	
	900	29.6	83	0	100	55	100	100	24	100	100	47.6	
Aniline	600	20	61.6	10	90	63.3	90	83.3	1.6	90	83.3	9.6	Normal stand of the weed and grasses
	750	20	70	10	96.6	81.6	96.6	96.6	3.3	96.6	96.6	16.6	
	900	21.6	86.6	13.3	100	96.6	100	100	7	100	100	26.6	
Terralin	600	16.3	11.6	0	33.3	0	33.3	0	3.6	33.3	0	4.6	Normal stand of the weed and grasses
	750	20	16.6	0	51.6	0	51.6	0	5.6	51.6	0	7.6	
	900	22.3	21.6	0	56.6	0	56.6	4.6	4.3	56.6	4.6	8	
Cresote	600	28.6	6.6	0	23.3	0	35	0	10	36.6	0	13.3	Normal stand of the weed and grasses
	750	29	11.6	0	41.6	0	55	0	15.6	55	0	19.6	
	900	30.6	18.3	0	45	0	65	8.3	20.6	68.3	8.3	20.3	

A supplementary experiment was carried out to determine the residual toxicity of the two effective chemicals in the soil 12 months after treatment. This was done by determining the viability of the Canada thistle roots extending into the treated plots from the adjacent untreated areas. The condition of the roots in successive two-inch layers is given in Table IV. It is evident

TABLE IV

RESIDUAL EFFECT OF CHEMICALS IN SOIL TWELVE MONTHS AFTER TREATMENT, JUDGED FROM VIABILITY OF CANADA THISTLE ROOTS EXTENDING INTO TREATED PLOTS FROM UNTREATED AREAS (Saskatoon series)

Depth examined	Condition of roots	
	Sodium chlorate plots	Arsenic pentoxide plots
In.		
0 - 2	Dead	Dead
2 - 4	Dead	Dead
4 - 6	Dead	Mostly fresh
6 - 8	Dead	Fresh
8 - 10	Dead	
10 - 12	Alive, but injured	
12 - 14	Fresh	

that sodium chlorate was still present in sufficient quantity in the first 10-in. layer of soil to kill roots penetrating that layer. In the plots treated with arsenic pentoxide this toxic condition of the soil was observed only in the first four inches. The most significant point is that both the effective chemicals had rendered the soil sufficiently toxic to prevent growth in the surface layer 12 months after treatment. Effects of this sort would of course be expected to vary with the character of the soil and rainfall.

Discussion and Conclusions

These results are of interest in connection with the methods employed for estimating the efficacy of perennial herbicides under field conditions. It has already been pointed out (3) that different investigators have made their final observations on the treated areas at various times after treatment. Some judge the efficacy shortly afterwards, others after longer periods, but during the same growth season, and still others have based their conclusions on the condition of the treated area a year or so later. It would appear that, until more information is available, the last is the only reliable criterion. The results presented in the foregoing tables show that some chemicals kill the herbage slowly, while others cause immediate death. The apparent mortality in such cases is therefore a function of time, and observations made within a month or two of treatment may lead to erroneous conclusions, since some of the slow-killing chemicals have the greatest permanent effect. Again two substances may kill the original herbage, but one may allow new growth to develop while the other may not. Judging from the results obtained in these experiments a chemical that kills the original herbage and does not allow

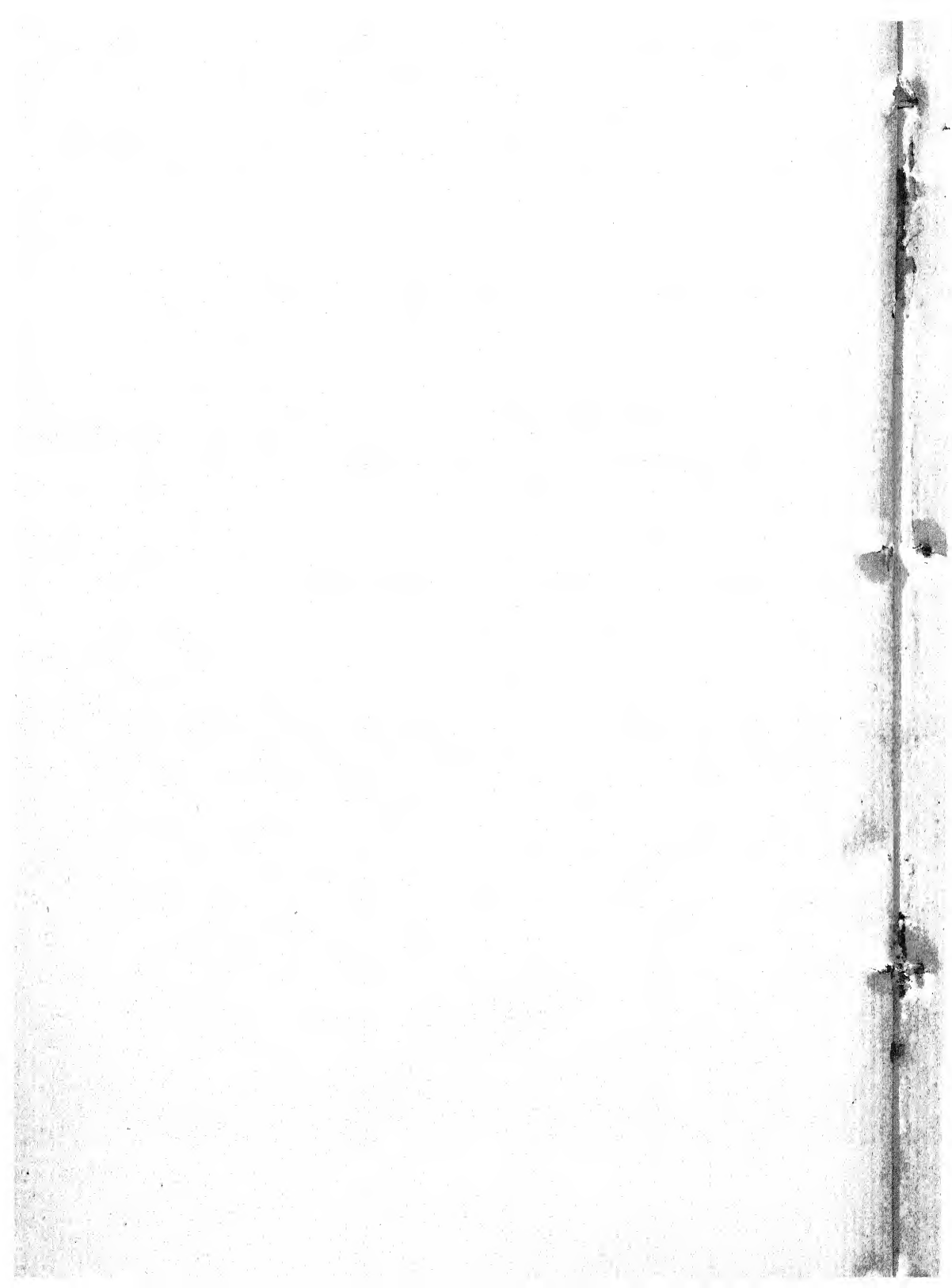
new growth to develop or survive during the season of treatment, will generally be found reasonably effective, as judged by the number of plants present the next season. However, a period of at least two months must elapse in order to permit new growth to develop.

The results show that a substance of high inherent toxicity may kill the aerial parts of the plant without having any significant effect on the roots. The experiments at Saskatoon indicate that the death of the roots depends on the presence of the chemical in the soil, and if the chemical is detoxicated by the soil before the roots are dead, the treatment is ineffective.

Of the 15 substances tested, only five appear to have a useful toxicity, namely, sodium chlorate, barium chlorate, arsenic pentoxide, ammonium thiocyanate and sodium arsenite. Sodium chlorate was most toxic, the certainly lethal dose (C.L.D.) under the conditions of these tests being about 600 lb. per acre. Barium chlorate and arsenic pentoxide are probably equally toxic, the C.L.D. being about 900 lb. per acre. Ammonium thiocyanate and sodium arsenite are less toxic than the others, but the C.L.D. cannot be given from the results obtained although it is probably in excess of 1200 lb. per acre. The relative toxicity of the effective chemicals, as judged from the C.L.D. is, therefore: sodium chlorate : barium chlorate and arsenic pentoxide : ammonium thiocyanate and sodium arsenite : as 1 : 1.5 : >2. It is possible that some of the other substances would effect mortality at much higher dosages, but unless they can be obtained at very low cost, they would not be practical herbicides. The results do not permit any statement to be made with respect to the relative susceptibility of the two species to applied chemicals.

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CHEMICAL WEED-KILLERS

IV. RELATIVE TOXICITIES AND LOCI OF ABSORPTION OF SELECTED CHEMICALS APPLIED TO PERENNIALS¹

By W. H. COOK²

Abstract

Twelve chemicals previously found to be highly toxic to annual weeds were applied to a perennial weed in three different ways, *viz.*, to the foliage only, to the soil only, and to both the soil and foliage. The chlorate ion was found to be most toxic, but sodium selenite, ammonium thiocyanate, sodium dichromate, and sodium arsenite were all reasonably effective at higher dosages. None of the other chemicals caused any significant, permanent reduction in growth at the dosages used. The permanent effect of a treatment appears to be due almost entirely to the action of the chemical in the soil, and the ineffectiveness of certain chemicals can be attributed to their rapid detoxication by the soil. Although all the chemicals exert a temporary, and in some cases, a slight permanent effect, when applied to the foliage only, this method of application is generally ineffective owing to the inability of the leaves and stems to retain or absorb a lethal dosage.

Introduction

This investigation was undertaken to obtain further information on the relative toxicities of certain chemicals to perennials, and also to determine whether these substances, when applied as a spray, entered the plant through the foliage, the roots, or through both these loci. The chemicals were selected from those previously found to be most toxic to annuals (2), but a number of them were not included since they had been found to be ineffective in field tests on perennials (4).

Materials and Methods

For the most part these experiments were made in an ordinary greenhouse in the same way as the previous tests on annuals (2). Perennial sow thistle, *Sonchus arvensis* L., was the only weed used. The plants were grown from root cuttings planted in the experimental crocks, and allowed to develop for a year before the various treatments were made. During this period the plants were cut back to prevent flowering, and fertilized several times. Before treatment all the weeds had a well developed root system which extended throughout the soil in the crock. The number of stems in each crock at the time of treatment varied from 2 to 5, the majority of plants having 4; the height also varied from 3 to 9 in. but a height of 6 to 7 in. was usual. The

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actual amount of foliage per crock was much more uniform than either the number of plants per crock or their height, because a reduction in number of plants per crock was compensated for by increased size of individual plants. To obviate any possible systematic error due to variable size, the crocks were distributed at random among dosages, treatments, and chemicals, and all phases of the experiments made concurrently.

Four dosages of each chemical were applied in all treatments, namely, 260, 520, 1050 and 2100 lb. per acre. The concentration of the solution sprayed on the plants was varied so that all these doses were applied in a volume of solution equivalent to 1050 gal. per acre. This procedure was followed so that the quantity of chemical retained by the foliage would be approximately proportional to the total dosage applied (3). This was important since three modes of application, *i.e.*, to the foliage only, to the soil only, and to both loci, were to be used. Ten of the 12 chemicals studied were applied in this way, namely, chloric acid, sodium chlorate, arsenic pentoxide, sodium arsenite, ammonium thiocyanate, sodium cyanide, copper nitrate, sodium selenite, sodium dichromate and phenol. Sodium sulphide and formic acid were applied only by the regular spray technique, *i.e.*, to both loci at once, to determine their relative toxicity.

The application of the toxic solution to the foliage only was accomplished by covering the surface of the soil with a coating of paraffin wax before spraying by the usual procedure. The amount of solution that drained to this wax covering was then collected and the quantity retained by the leaves estimated from the difference between the applied and recovered volumes. The retained volume varied with the size of the plants from $\frac{1}{8}$ to $\frac{1}{4}$ of the volume applied. Any chemical remaining on the wax covering was washed off two days later and the wax removed. Applications to the soil only were made with a pipette, the solution being well distributed over the surface. The usual spraying procedure was used to apply the toxic solution to both the foliage and soil. Two types of controls were used, the original plants being allowed to grow in one set, while in the other they were cut back to the soil level at the time of treatment in order to produce conditions comparable with chemical applications which affect the foliage only. Each treatment of whatever kind was made in duplicate, with an additional number of controls.

The plants were treated in the middle of May. Periodic observations were made thereafter to determine the mortality of the original foliage and the time required for new plants to develop. The final observations were made four months after treatment, when the number and wet weight of the surviving plants (stems and leaves) were determined. Dead stems and tissue were removed before weighing the living material. This could be done quite accurately in these tests, since the elapsed time from treatment and death of the original stems was of sufficient duration to permit precise distinctions. One week later the crocks were seeded to wheat to determine whether any residual toxic effect persisted in the soil.

Results

OBSERVATIONS FOLLOWING TREATMENT

Of the observations taken between the time of treatment and the final quantitative determinations, the times required for the death of the leaves on the original plants, and for the development of new plants, appear to be the most valuable. These results are summarized in Table I, the chemicals being arranged in groups in order of toxicity. While the lowest dosages were generally least effective (see Table II) and acted more slowly than the higher dosages, the rates of action were not sufficiently different to justify detailed consideration. Some idea of the variability can be gained from the range of values reported in Table I. It was observed that, if a long period was required

TABLE I
DAYS FROM TREATMENT UNTIL DEATH OF LEAVES AND APPEARANCE OF NEW PLANTS

Chemical	Application to foliage only		Application to soil only		Application to both foliage and soil	
	Days for death of leaves	Days for appearance of new plants	Days for death of leaves	Days for appearance of new plants	Days for death of leaves	Days for appearance of new plants
Sodium chlorate	30-40*	10	21-60	20-69	32-64	65-75
Chloric acid	2-3	5-6	25-50	60†	2-3	3-9
Sodium selenite	3	7-10	90*	30-90†	3	5-10†
Ammonium thiocyanate	3	7-10	7-28	7-28	4-5	5-10†
Sodium dichromate	2	7	10-50	15-63†	2-4	7-40†
Sodium arsenite	3-4	10-12	6-10*	14-30	4	4-20
Sodium cyanide	3	7-14	7-10*	10-50	3	7-10
Arsenic pentoxide	3	7-14	No effect beyond wilting		3	3-14
Copper nitrate	2	6-8	No effect beyond slight wilting		2	6-8
Phenol	2-4*	6-8	No effect beyond slight wilting		2-4*	8-30
Sodium sulphide	-	-	-	-	3*	14-30
Formic acid	-	-	-	-	1-2	2-3

* Leaves and stems revived at low doses.

† No new growth at high doses.

for the death of the original foliage, the development of new plants was also slower, indicating that the plant tends to maintain the original foliage at the expense of new plant development. Quantitative information was obtained only from the final determinations, which will now be considered.

FINAL DETERMINATIONS

The number and weight, of plants in each crock four months after treatment were the two criteria used to estimate the efficacy of the treatment. These results are given in Table II. Before discussing them, the relative value of the two criteria must be considered, for, although they generally

agree, there are some cases of divergence. Mortality is the criterion commonly used for measuring toxicity, and comparisons of the number of plants in the treated and untreated crocks probably give the best estimate of this quantity. At the same time it must be recognized that this is not a per-

TABLE II
EFFECT OF CHEMICAL AND METHOD OF APPLICATION ON NUMBER AND GREEN WEIGHT
OF LIVING STEMS AND LEAVES

Chemical and dosage, lb. per acre	No. of plants per crock			Wet wt. of plants per crock		
	Mean of duplicates			Mean of duplicates		
	Application to foliage only	Application to soil only	Application to foliage and soil	Application to foliage only, gm.	Application to soil only, gm.	Application to foliage and soil, gm.
Sodium chlorate						
260	11.5(1) *	1.5	1	86.5(1)	15.0	7.0
520	7.5	0	0	85.0(1)	0	0
1050	10.0(1)	0	0	55.5	0	0
2100	1.0(2)	0	0	12.0(2?)	0	0
Chloric acid						
260	13.5(1)	2.0	5.0	55.5	13.5	43.0
520	2.0(2)	0.5	0	39.0	1.5	0
1050-2100	0	0	0	0	0	0
Sodium selenite						
260	13.5(1)	5.0	9.0	98.5	76.0	77.0(1)
520	2.5(2)	2.5	3.5	96.0	42.0	76.5
1050	7.0	3.5	0.5	66.5	25.0	14.5(2)
2100	5.0	0	0	71.5	0	0
Ammonium thiocyanate						
260	8.0	6.0	9.5	48.0	52.5	107.0(1)
520	13.0(1)	11.5(1)	8.0	104.0(1)	73.5(1)	58
1050	12.5	0.5(2)	1.0	108.5(1)	5.0(2)	27 (2)
2100	2.0(2)	0	0	38.5(2)	0	0
Sodium dichromate						
260	13.0(1)	9.0	13.5(1)	71.5	62.5	82.5
520	11.5	1.0	6.0	100.0(1)	37.0	81.0
1050	10.0	6.0	0.5(2)	80.0	89.5	32.0
2100	4.0(2)	0	0	32.5(2)	0	0
Sodium arsenite						
260	12.0	8.5	6.0	76.5	75.5(1)	97.5(1)
520	13.5	6.5	2.0	61.5	85.0(1)	61.5
1050	11.0	2.5	0	85.0	58.5	0
2100	12.0	0.5	0.5	46.0	1.5(2)	12.5(2)
Sodium cyanide						
260	19.5(1)	12.5(1)	13.0(1)	83.5	89.0	85.0
520	6.5(2)	8.5	12.5(1)	77.5	82.0	77.0
1050	12.0	5.0	5.0	97.5	59.5	61.0
2100	16.5	1.5(2)	2.5 (2)	77.5	33.5	25.5
Arsenic pentoxide						
Mean of all 4 doses	3.4	8.0	3.6	70.0	95.4†	42.5†

* Quantities marked (1) are significantly different from quantities marked (2) for same chemical and mode of application.

† These two significantly different.

TABLE II—*Concluded*
EFFECT OF CHEMICAL AND METHOD OF APPLICATION ON NUMBER AND GREEN WEIGHT
OF LIVING STEMS AND LEAVES—*Concluded*

Chemical and dosage, lb. per acre	No. of plants per crock			Wet wt. of plants per crock		
	Mean of duplicates			Mean of duplicates		
	Application to foliage only	Application to soil only	Application to foliage and soil	Application to foliage only, gm.	Application to soil only, gm.	Application to foliage and soil, gm.
Copper nitrate Mean of all 4 doses	12.4	18.8	13.6	77.0	92.4	88.9
Phenol Mean of all 4 doses	11.2	12.6	13.4	80.2	77.6	91.4
Sodium sulphide Mean of all 4 doses			12.6			77.6
Formic acid Mean of all 4 doses			10.6			93.8
Controls Cut back Allowed to grow	18.7(1) 8.0(2)			77.6 (Mean of both types of controls as not significantly different.)		

* Quantities marked (1) are significantly different from quantities marked (2) for same chemical and mode of application.

centage mortality comparable with that obtained with organisms in which the final effect can be observed within a short period after treatment. During the period allowed between treatment and the final count, the number of plants in the cut and uncut controls increased from an average of 3.6 plants per crock to 18.7 and 8.0 respectively. This shows that growth during this period introduces one complication. In the treated crocks this complication becomes more serious; a given poison might cause high mortality in the original plants, including the roots, but if the chemical is detoxicated in the soil, a small amount of living root material might re-establish the original number of plants before the final observations are made. This behavior is believed to account for the high variability observed between duplicates in these experiments. Finally, any estimate of the number of plants of a perennial weed is really an estimate of the number of stems, many of which may come from a common root. These may correctly be termed plants if they can exist separately, but they might not behave as such toward an applied chemical.

From a practical standpoint the size and number of weeds per unit area, and their rate of re-establishment, determine their competitive influence on economic crops. The number alone is of little value, for if only a few plants survive they may be large, and *vice versa*. It appears, therefore, that a

practical measure of efficacy, such as the weight of weed material, is the best criterion of toxicity to use, until more is known about the dosage-mortality relations of poisons and perennial weeds.

An initial study of the weight of the plants was made by computing the averages obtained from all crocks at all dosages for each chemical and method of application, and studying the results statistically. Taking a 5% level of significance, it was found that the chemicals could be classified into four distinct groups which differed significantly from one another. Sodium chlorate and chloric acid caused the greatest reduction in weight and constituted the first group; sodium selenite, ammonium thiocyanate, sodium dichromate and sodium arsenite the second; sodium cyanide and arsenic pentoxide the third, which did not differ significantly from the controls; and sodium sulphide, copper nitrate, phenol and formic acid the last group, in which the weight of plant material was significantly greater than in the controls, suggesting direct or indirect stimulation or nutritive effects. Application of the chemical to the foliage or soil only, or to both loci, gave essentially the same grouping.

Since all the chemicals were applied at the same dosages, such an analysis is useful for comparative purposes, but it gives no information as to the effect of different dosages. These more detailed results are given in Table II, where the chemicals are again arranged in order of decreasing toxicity, although the differences are significant only between the groups indicated. Sodium chlorate appears first and, although it did not differ significantly from chloric acid, the figures suggest that it is slightly less toxic than the latter. It must be remembered, however, that, on the basis of chlorate ion, the applied dosage of sodium chlorate was only 78% of that applied as chloric acid.

The first six chemicals caused complete mortality at one or more of the higher dosages. The certainly lethal dose gives an estimate of their relative toxicities, but the data do not permit evaluation of the extent to which these lethal dosages may be in error. Where a given treatment did not give a complete kill, but the number or weight of plants differed significantly between doses, the results are given, and the quantities that differ significantly are indicated. Owing to the large variability between duplicates, only relatively large differences were significant. Where the weights of the plants did not differ significantly, and the significant differences between the number of plants did not appear to be related to the dosage, the mean values at all dosages are the only figures reported. Actually the significant differences between the numbers of plants in crocks receiving different dosages of copper nitrate suggested stimulation with increasing dosages, while phenol appeared to be the most effective as a poison at intermediate doses.

It is evident that the first six chemicals were about equally effective whether applied to the soil only, or to the foliage and soil, but were comparatively ineffective when applied to the foliage only. The significant differences between dosages applied only to the leaves indicate that, with the possible exception of sodium arsenite, all the chemicals can bring about a permanent reduction

in growth through the foliage but are comparatively ineffective owing to the inability of the leaves to retain or absorb a lethal quantity. The efficacy of sodium chlorate applied to the leaves must also be questioned, since a slight residual effect, discussed in the next section, was evident in one of the crocks, indicating that some of the chemical might have entered the soil through the wax. It should also be noted that chloric acid was comparatively more effective than the other chemicals when applied to the foliage only.

The remaining chemicals produced no evidence of a permanent effect and consequently showed little difference between the three methods of application. Arsenic pentoxide, applied to the soil only, gave significantly higher plant weights than applications to foliage and soil. Neither treatment differed significantly from the controls, so that the above results must be attributed to stimulation following applications to the soil, and a slight toxic action when applied to both foliage and soil.

RESIDUAL EFFECT IN SOIL

After the final determinations were made on the weeds, the crocks were seeded with wheat to determine whether there remained a residual toxic effect in the soil. The final observations on these plants were made 45 days after seeding. In general, only crocks that had received the two highest dosages were used. Crocks representing each method of application of sodium chlorate and chloric acid were tested, since these chemicals were effective when applied by all three methods. Crocks, in which the other chemicals had been applied to the foliage only, were excluded, since such treatments were ineffective. Crocks treated by all methods with copper nitrate, phenol, sodium sulphide, and formic acid were excluded for similar reasons.

The results will not be presented in detail, but are summarized below:—

Sodium chlorate. Applications to soil showed definite evidence of residual chemical, the plants being small and weak. Applications to the foliage only left no residual effect, except in one crock that received the maximum dosage, in which the plants were somewhat smaller than in the control crocks.

Chloric Acid. Applications to soil left a residual effect that increased with applied dosage. No effect was evident following applications to foliage only.

Sodium selenite. Plants died within three weeks in crocks that had received the two highest dosages applied to the soil. Applications to foliage and soil also left a marked residual toxic effect, which did not result, however, in complete mortality.

Sodium arsenite. Only slight evidence of residual chemical showed in crocks that had received the two highest dosages applied to the soil; similar dosages applied to both foliage and soil had no apparent residual toxic effect.

Arsenic pentoxide. Only slight evidence appeared of residual chemical following applications of maximum dosage to foliage and soil.

Ammonium thiocyanate, *sodium dichromate*, and *sodium cyanide*. No evidence was found of any residual chemical in the soil.

This summary shows that the chlorate and selenite ions have a decided residual effect. The fact that sodium selenite has less effect than the chlorates on the growth of thistles, and an equal or greater effect on the growth of wheat planted subsequently, must be attributed to a difference in the susceptibility of sow thistle and wheat to this chemical. A difference in the susceptibility of these two plants to arsenicals would also explain the residual toxic effects observed with wheat in crocks that had previously supported considerable growth of thistles. Substances that killed the thistles but showed no residual effects must have been detoxicated before the wheat was planted, at least to a point below that at which wheat was affected.

Discussion and Conclusions

All the chemicals tested, with the exception of sodium chlorate, killed the leaves of the plants within a few days after treatment when applied directly to the foliage; four months later, however, reduction of the weed growth was apparent for only 6 of the 12 chemicals used. This shows that the mere destruction of the foliage is no indication of the efficacy of a herbicide. Of the six effective chemicals, sodium chlorate and chloric acid were significantly more toxic than sodium selenite, ammonium thiocyanate, sodium dichromate and sodium arsenite. The certainly lethal dose of the last chemical might be higher than the maximum dose employed, but it reduced growth to such an extent that, on the basis of weight, its toxicity did not differ significantly from that of the other chemicals in the second group.

In general, the toxic chemicals were equally effective whether applied to the soil only or to the foliage and soil, but were comparatively ineffective when applied to the foliage only. The latter result is attributable to the inability of the aerial parts to retain or absorb more than a small proportion of the applied dosage. Chloric acid was the only exception to this rule, for although applications to the foliage only were generally least deadly, this method of application proved to be relatively more effective with this chemical than with the other chemicals tested. These results show that the observed reduction in the number and size of perennial plants, following the application of a herbicide as a spray, is due mainly to the action of the chemical in the soil, rather than to its direct effect on the leaves.

All the chemicals used in these experiments were inherently quite toxic to plants as judged from their effect on annual weeds (2) and on the foliage of perennials. Since the mortality of perennials is due, however, almost entirely to the presence of the poison in the soil, it follows that the detoxicating effect of the soil, rather than the inherent toxicity of the chemical, is the factor limiting the efficacy of certain substances. The permanent effect of chemical sprays will depend, therefore, on the relative rates of poisoning and detoxication and, if the latter reaction is rapid compared with the former, the treat-

ment will be ineffective. Some estimate of the rate of poisoning can be obtained from the time required to kill the leaves, following applications to the soil only. This period varied from 10 to 60 days, and, in general, the slowest acting chemicals proved to be most effective. Certain chemicals did not kill any of the leaves, when applied to the soil, indicating that these substances were detoxicated within a relatively short period. It appears, from these considerations, that the observed variability in the relative toxicity of different chemicals to perennials is due mainly to variable detoxication by the soil rather than to variable susceptibility of the plants grown under different conditions. A herbicide which is consistently effective under all conditions will, therefore, be one which is not readily detoxicated by any soil, and will remain effective for a period of 60 to 90 days until all plant roots are dead. The efficacy of chlorates can be explained on this basis, since they are apparently not detoxicated as rapidly by different soils as certain other compounds used as herbicides (1, 5). Arsenicals, although they are inherently quite toxic, are apparently susceptible to detoxication in certain soils, at least to a level where they are ineffective against perennials. Thus arsenic pentoxide appeared to be quite effective in field tests (4) but was quite useless in these experiments.

If the efficacy of a given perennial herbicide is to be rendered independent of the soil, some consideration should be given to the possibility of poisoning the plants through the leaves. Furthermore, since the efficacy of chemicals acting through the soil is at least reduced by dilution, if not by detoxication, it seems probable that the certainly lethal dose of a given chemical would be lower if it could be made to act through the leaves rather than through the soil. The comparative ineffectiveness of applications to the foliage only in these experiments can be attributed entirely to the inability of the leaves and stems to retain or absorb a lethal dosage, since in no case was more than $\frac{1}{4}$ of the applied dosage retained by the leaves and stems. At the same time the number and weight of plants was reduced significantly in several cases as the dosage, *i.e.*, concentration, applied to the foliage only, was increased. This suggests that the chemical can act through the leaves and might be effective if retained and absorbed by the foliage in sufficient quantity. If this is so, the quantity retained could be increased by increasing the concentration of the spray solution (3) within the limits of the solubility of the chemical. The results obtained with chloric acid suggest that an acid condition favors absorption.

In conclusion it is worth noting again that with the composition of the solutions commonly applied to perennials as herbicides, the permanent lethal effect observed comes almost entirely from the action of the chemical in the soil. Under these conditions any system of sprinkling or distributing the solution over the soil area should prove effective. For annual weeds, however, where the comparatively much smaller dosages required act almost entirely on the foliage, a good distribution of the solution over the leaves is essential.

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AGRICULTURAL METEOROLOGY: SOME CHARACTERISTICS OF AIR TEMPERATURE IN ALBERTA AND SASKATCHEWAN¹

By J. W. HOPKINS²

Abstract

A five-year moving average of mean temperature for the period April 1 to August 31 has a range of variation of about 4° F. over the years 1893-1933 at Edmonton and Calgary (central and southern Alberta), and Battleford and Swift Current (central and southern Saskatchewan). The general trend over the 41 years is slightly upward at the last three stations; there are also shorter irregular cycles of above- and below-average values of both mean temperature and mean daily range at all four stations. Short-term fluctuations of the former are most pronounced in April and May; those of the latter are equally in evidence in all five months. The annual averages of daily mean temperature (April 1-August 31) tend at all four stations to fall more frequently above than below the general average for the 41 years. Annual variation in monthly mean temperature is greatest in April and least in July, but the mean daily range is as variable for the summer as for the spring months. There is a fairly close correlation between the annual variations in mean monthly temperature at the four stations, but no significant association between the mean temperature of successive months at the same station. Below-average monthly temperatures tend to be associated with above-average precipitation, particularly in July and August.

During 1916-1933, the intra-monthly variation of both daily maximum and minimum is greatest in April and least in July and August. Within any of the five months, the daily maxima are more variable than the minima. There is a significant correlation between the daily maxima and minima within months (which however diminishes progressively from April to August), and between the mean maximum and mean minimum of April, May and August in different years. The frequency distributions of the 18 years' daily maxima and minima have individual characteristics for each of the five months, which show a generally similar seasonal trend at the four stations. Seasonal trend in the daily range is much less than that in the mean temperature.

The hourly temperature at Swift Current, averaged over four years, attains its maximum at 3 p.m. in all five months studied. The hour of average minimum varies from 6 a.m. in April to 4 a.m. in June. In each month the hours with below-average are in excess of those with above-average temperatures, but this inequality is more pronounced in spring than in summer. The mean of the daily maximum and minimum may sometimes deviate considerably from the mean of the 24 hourly observations.

In an earlier communication (4), the writer discussed certain features of the precipitation statistics for selected meteorological stations in central and southern Saskatchewan and Alberta. Observations of air temperature during the months of the growing season, recorded at these stations and published by the Meteorological Service of Canada (7) are now examined in a similar manner.

The temperature data in question were recorded by thermometers placed in the usual standard screens at a specified height above ground level. It is true that from the agricultural point of view, such data are in some respects inadequate, and may be usefully supplemented by the readings of instruments freely exposed at various levels (3, 9, 10). They do, however, provide a comparable series of standard observations, characteristic of each station.

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Annual Variation in Seasonal and Monthly Averages

A study was made of the annual variations in air temperature at four stations, namely, Edmonton (lat. $53^{\circ} 33' N.$, long. $113^{\circ} 30' W.$, alt. 2158 ft.) and Calgary ($51^{\circ} 2' N.$, $114^{\circ} 2' W.$, 3428 ft.) in central and southern Alberta respectively, and Battleford ($52^{\circ} 41' N.$, $108^{\circ} 20' W.$, 1592 ft.) and Swift Current ($50^{\circ} 20' N.$, $107^{\circ} 45' W.$, 2392 ft.) in central and southern Saskatchewan.

SECULAR TREND

An examination of long-time records from various parts of the world made by Kincer (6) led him to the interesting conclusion that the trend of mean annual temperature in the middle latitudes of the northern hemisphere has been definitely upward for more than half a century. The American data (from eastern and mid-western stations) further indicated that the upward trend in the annual means was largely due to a progressive moderation of autumn and winter temperatures, and to a smaller extent to a rise in those for the spring period. During the summer, on the other hand, variations

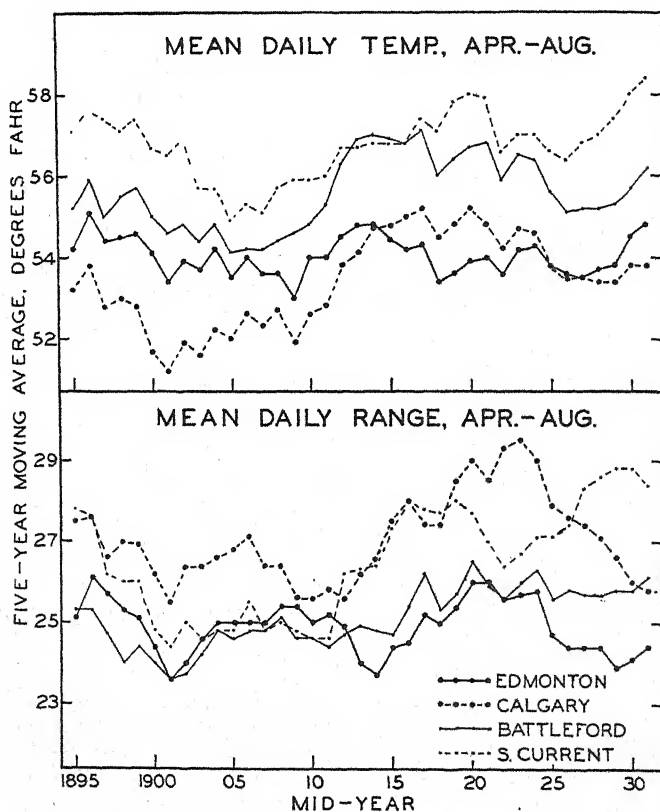


FIG. 1. Five-year moving average of mean-temperature (upper portion) and mean daily range (lower portion) April 1 to August 31, at Edmonton (central Alberta), Calgary (southern Alberta), Battleford (central Saskatchewan) and Swift Current (southern Saskatchewan), 1893-1933.

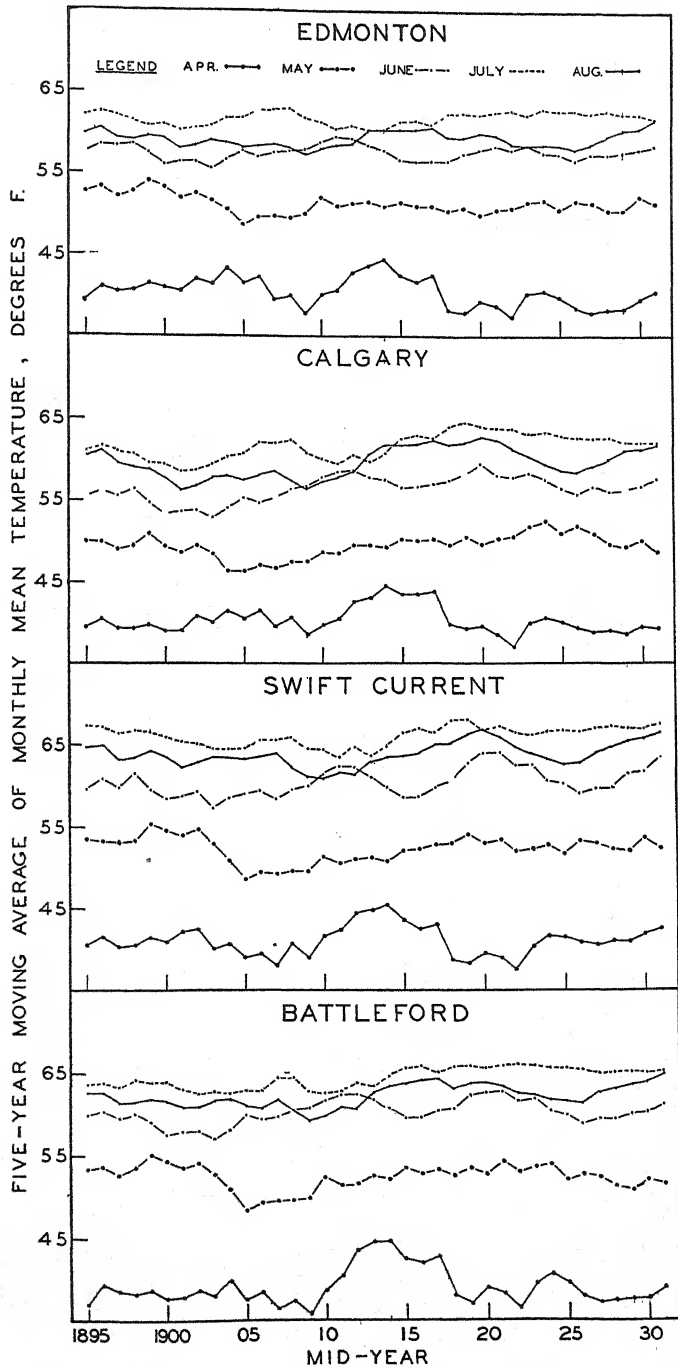


FIG. 2. Five-year moving averages of monthly mean temperature at Edmonton (central Alberta), Calgary (southern Alberta), Battleford (central Saskatchewan) and Swift Current (southern Saskatchewan), 1893-1933.

in the 20-year moving average employed by Kincer were for the most part confined to alternating intervals, of irregular length, above and below the average for the entire period covered by the records, this season contributing least to the long-time trend.

Fig. 1 (upper portion) shows the course of a five-year moving average of mean temperature (average of daily maxima and minima) for the spring and summer months, April 1 to August 31, at the above four Canadian stations over the years 1893 to 1933. The moving average has a rather narrow range of variation (about 4° F.) at each station, but within these limits both short- and long-term variation with time is discernible. Over the 41-year period as a whole, the general trend is slightly upward at Calgary, Battleford and Swift Current. At Edmonton, on the other hand, the short-term alternations seem to have taken place about a very stable long-time mean.

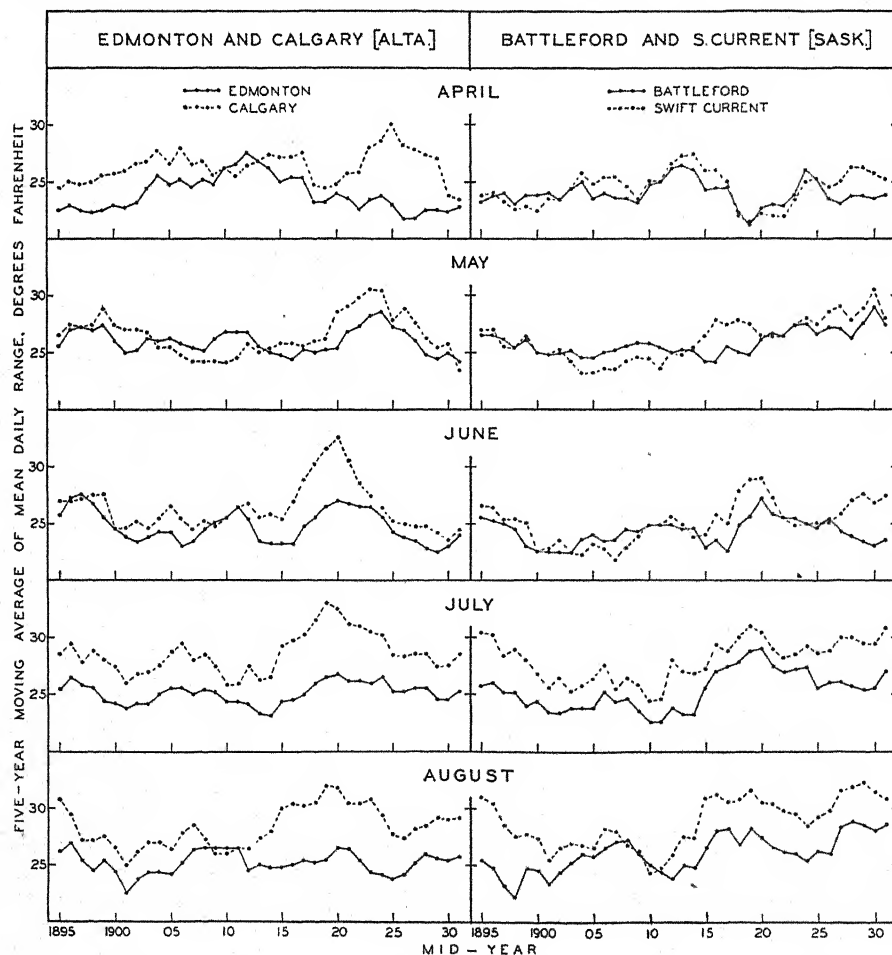


FIG. 3. Five-year moving averages of monthly mean daily range in temperature at Edmonton (central Alberta), Calgary (southern Alberta), Battleford (central Saskatchewan) and Swift Current (southern Saskatchewan), 1893-1933.

The short-term variations are irregular in both phase and amplitude, but it is possible nevertheless that they may be connected to some extent with the rather complex fluctuations in the constant of solar radiation, recently described by Abbott (1).

The lower portion of Fig. 1 shows the course of a similar five-year moving average of the mean daily range in temperature for the same five-month period. This quantity proved to have been slightly more variable than the mean temperature itself, the maximum range of fluctuation of the moving average at an individual station being 5° F. Here also there is some indication of short-term variation, three irregular cycles of high and low values being traversed during the 41-year period.

Fig. 2 shows the five-year moving averages of mean temperature for the individual months. At all four stations, the moving averages for April and May show the most pronounced short-term fluctuations. In June, July and August such recurrent oscillations are smaller (moreover, those for each month seem to be largely independent) but the indication of a slight long-time trend is more definite, particularly at Calgary and Swift Current.

A different situation prevails in the case of the monthly mean daily range. At each station the moving average of this quantity shows variations with time in all five months, illustrated in Fig. 3, but the short-term fluctuations are just as pronounced in summer as in spring, if not more so.

FREQUENCY DISTRIBUTION OF SEASONAL AND MONTHLY AVERAGES

As only 41 years' observations were available, the characteristics of the frequency distribution of the annual values could not usefully be examined in any detail.

Table I (left portion) shows the distribution, between two-degree class intervals, of the four series of 41 annual values of mean temperature for the five-month period April 1–August 31. At all four stations, the annual five-month averages tend to be concentrated about certain modal values, rather than to occur with approximately equal frequency over the whole range of variation. There is also at all stations a disposition towards asymmetry in the frequency distribution, a majority of the observations falling in the upper part of the range of variation. The general level of April–August mean temperature is slightly higher at the more continental Saskatchewan stations than at the two Alberta ones. The effect of latitude may also be discerned in the observations at Battleford and Swift Current, but in the case of Edmonton and Calgary this factor seems to be more than offset by the difference in altitude. The total range of annual variation is seen to be greatest at Swift Current and least at Edmonton.

A similar classification of the mean daily range in temperature during the period April 1 to August 31 of each of the 41 years is shown in the right half of Table I. This quantity also exhibits modality and a tendency towards asymmetry of distribution, the lower values being on the whole the more

TABLE I

FREQUENCY DISTRIBUTION OF ANNUAL VALUES OF MEAN DAILY TEMPERATURE AND MEAN DAILY RANGE, APRIL 1-AUGUST 31, AT METEOROLOGICAL STATIONS IN CENTRAL AND SOUTHERN ALBERTA AND SASKATCHEWAN, 1893-1933

Mean daily temperature, April 1-August 31						Mean daily range, April 1-August 31					
Class interval, °F.	Edmonton	Calgary	Battleford	Swift Current		Class interval, °F.	Edmonton	Calgary	Battleford	Swift Current	
48.0 - 49.9		2				20.0 - 21.9	1				
50.0 - 51.9	4	7	1	1		22.0 - 23.9	11	3	12	6	
52.0 - 53.9	12	14	8	2		24.0 - 25.9	21	9	16	10	
54.0 - 55.9	21	14	11	9		26.0 - 27.9	6	14	12	15	
56.0 - 57.9	4	4	17	16		28.0 - 29.9	2	12	1	6	
58.0 - 59.9			4	11		30.0 - 31.9		3		4	
60.0 - 61.9				2							
41-year average, °F.	54.1	53.4	55.6	56.8		41-year average, °F.	24.8	27.0	25.2	26.6	

numerous. The 41-year average of the five-month mean daily range is higher at the two southern stations, Calgary and Swift Current, than at Edmonton and Battleford.

Fig. 4 shows the frequency distribution of the individual monthly means, the class interval being 4°F. for April and 2°F. for May, June, July and August. All the frequency distributions show some degree of modality, and the seasonal diminution in annual variability at each station from April to July is quite marked. This is the reverse of the situation with respect to monthly precipitation, which was least variable in April and fluctuated most from year to year in June and July (4). In all five months the annual variation is less at Edmonton (central Alberta) than at any of the other stations. Table II summarizes the average values and range of variation of the monthly means.

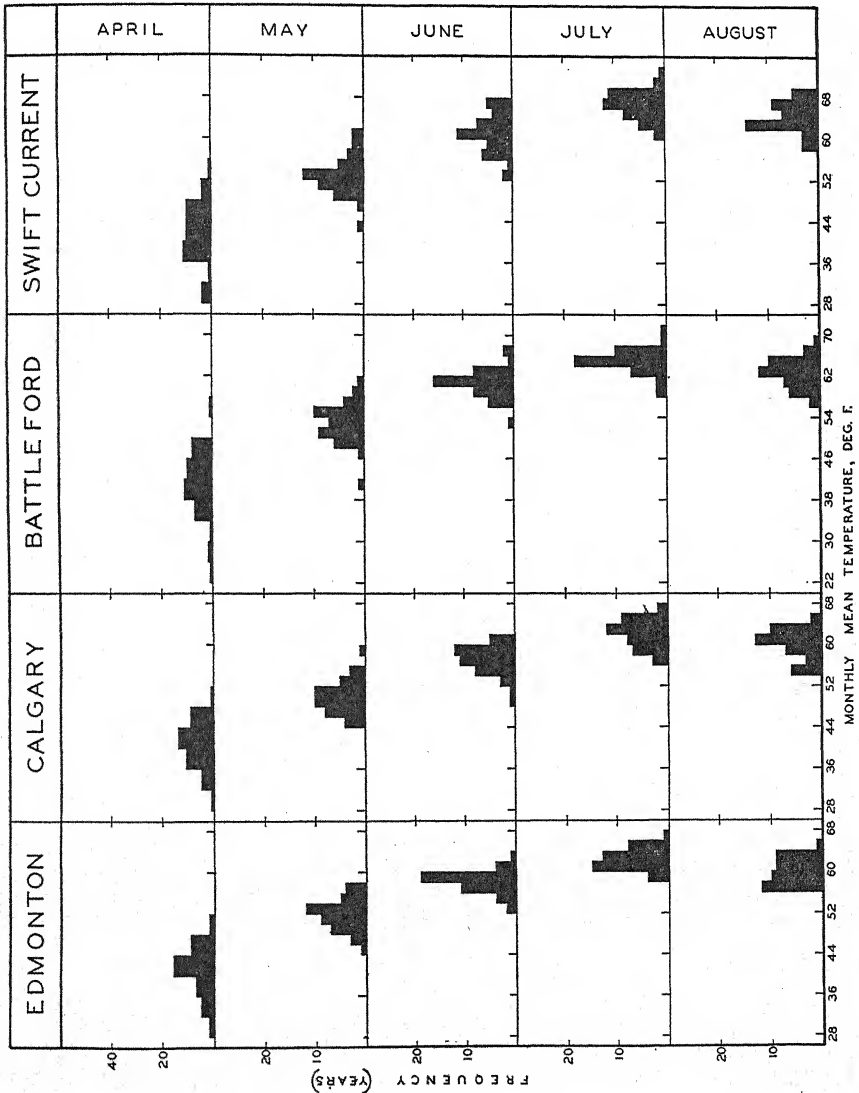


FIG. 4. Frequency distribution of monthly mean temperature at Edmonton (central Alberta), Calgary (southern Alberta), Battleford (central Saskatchewan) and Swift Current (southern Saskatchewan), 1893-1933.

TABLE II

MEAN DAILY TEMPERATURE (°F.) BY MONTHS AT METEOROLOGICAL STATIONS IN CENTRAL AND SOUTHERN ALBERTA AND SASKATCHEWAN, 1893-1933

Station	April		May		June		July		August	
	Average 1893- 1933	Max. Min.	Average 1893- 1933	Max. Min.	Average 1893- 1933	Max. Min.	Average 1893- 1933	Max. Min.	Average 1893- 1933	Max. Min.
Edmonton	40.3	49	51.3	57	57.5	62	61.8	66	59.4	65
Calgary	40.0	49	49.3	58	56.3	61	61.5	66	59.6	65
Battleford	38.8	52	52.3	60	60.1	66	64.4	70	62.3	69
Swift Current	41.0	52	52.3	60	60.5	66	66.2	72	64.0	68

TABLE III

MEAN DAILY RANGE IN TEMPERATURE (°F.) BY MONTHS AT METEOROLOGICAL STATIONS IN CENTRAL AND SOUTHERN ALBERTA AND SASKATCHEWAN, 1893-1933

Station	April		May		June		July		August	
	Average 1893- 1933	Max. Min.	Average 1893- 1933	Max. Min.	Average 1893- 1933	Max. Min.	Average 1893- 1933	Max. Min.	Average 1893- 1933	Max. Min.
Edmonton	23.8	32	25.8	32	24.7	31	25.1	29	25.2	32
Calgary	26.0	33	26.3	33	26.3	36	28.6	36	28.6	35
Battleford	23.8	30	25.9	31	24.4	31	25.6	32	26.3	32
Swift Current	24.3	32	26.2	36	25.3	33	28.3	36	29.0	36

A similar treatment of the monthly values of mean daily range is shown in Fig. 5 and Table III, the class interval in Fig. 5 being, however, 2° F. for all months. Here again values in the neighborhood of the average tend to be the more numerous, and the annual variation of the monthly means is

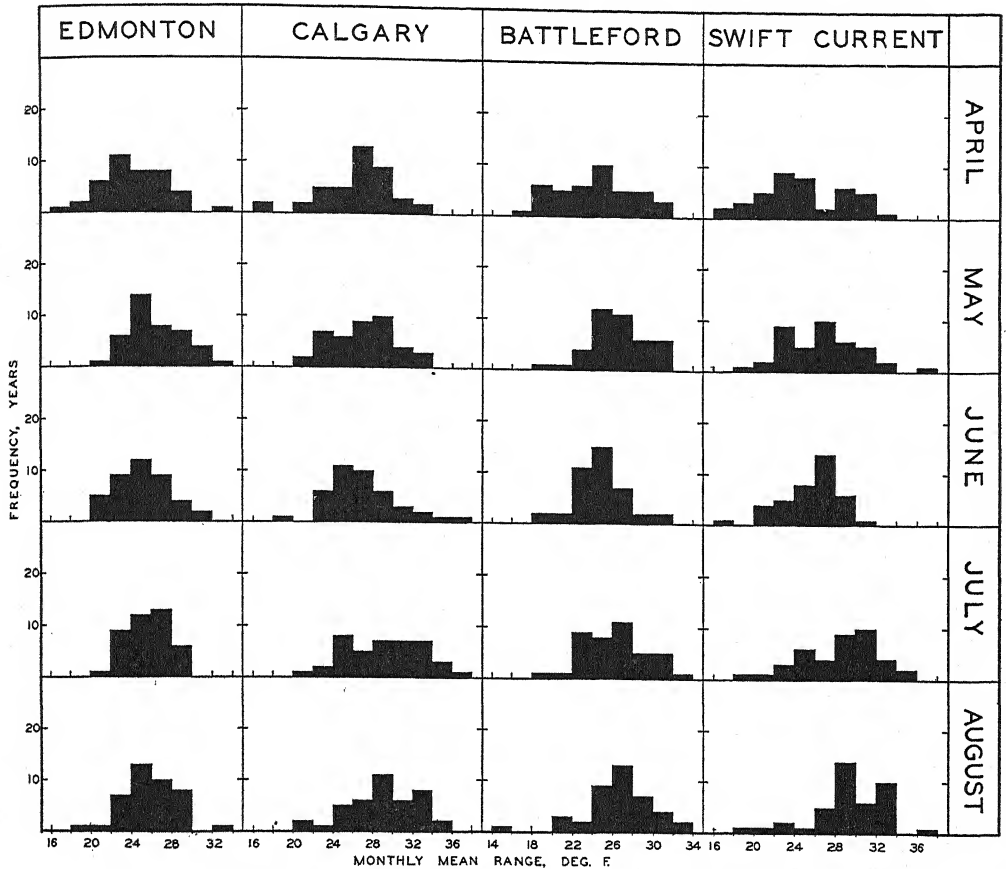


FIG. 5. Frequency distribution of monthly mean daily range in temperature at Edmonton (central Alberta), Calgary (southern Alberta), Battleford (central Saskatchewan) and Swift Current (southern Saskatchewan), 1893-1933.

on the whole least at Edmonton and Battleford and greatest at Calgary and Swift Current. The 41-year averages of mean daily range (Table III) are in general slightly higher for the summer than for the spring months. There is however no diminution of the annual variability of the values for the summer as compared to those for the spring months; in fact the former show, if anything, greater variation from year to year.

INTRA- AND INTER-MONTHLY CORRELATIONS

In the writer's previous study of the precipitation statistics for this area (4), it was found that there was a significant association in the total of spring and summer precipitation in the four districts, central and southern Saskat-

chewan and Alberta, but that the degree of correlation was only moderate ($r = 0.3$ to 0.5), leading to the conclusion that the simultaneous occurrence of extremely dry or wet seasons over the whole area may be expected to be infrequent. As anticipated, there is a considerably closer association between the annual variations in monthly mean temperature, all the inter-station coefficients in Table IV exceeding the 1% point. On the whole, inter-station

TABLE IV

INTER-STATION CORRELATION COEFFICIENTS (r) OF ANNUAL MONTHLY MEAN TEMPERATURE, 1893-1933

Stations	April	May	June	July	August
Edmonton X Calgary	0.92	0.78	0.82	0.80	0.81
X Battleford	.77	.86	.85	.55	.82
X Swift Current	.86	.81	.75	.63	.71
Calgary X Swift Current	.86	.75	.63	.74	.82
X Battleford	.88	.75	.87	.74	.82
Battleford X Swift Current	.91	.88	.81	.69	.84

Value of r at 5% point = 0.31.

Value of r at 1% point = 0.40.

correlation is greatest in April, the month of most pronounced variations in temperature from year to year of the five considered, and least in July, the month of highest mean temperature but lowest annual variability. In general the north-south correlations (Edmonton X Calgary and Battleford X Swift Current) are higher than the east-west ones.

The foregoing correlation does not however extend to the mean temperature of successive months at the same station, all the inter-monthly coefficients listed in Table V being small, only three out of 40 exceeding the

TABLE V

INTER-MONTHLY CORRELATION COEFFICIENTS (r) OF MONTHLY MEAN TEMPERATURE, 1893-1933

Months	Edmonton	Calgary	Battleford	Swift Current
April X May	0.20	0.28	0.17	0.23
X June	.13	.17	.11	.25
X July	-.12	.00	-.18	-.04
X August	.22	.12	.13	-.04
May X June	-.11	.02	-.04	.04
X July	-.10	.07	.05	.35
X August	.19	.19	.26	.25
June X July	-.14	.22	.15	.17
X August	-.02	.25	.07	.14
July X August	-.02	.39	.36	.22

Value of r at 5% point = 0.31.

Value of r at 1% point = 0.40.

5% point and none attaining the 1% point. It would seem therefore that over the 41-year period as a whole, the mean temperature of the five individual months fluctuated independently from year to year. This does not necessarily imply that an above-average mean temperature for June, for example, was never followed by an above-average mean for July, but does indicate that such situations did not occur more frequently than would be expected from chance combinations.

Association between the fluctuations from year to year of the precipitation and mean temperature of the same month was investigated by the calculation of the correlation coefficients shown in Table VI. As the study of rainfall (4) had already indicated that there was a correlation between total precipitation and the number of rainy days per month, and as on rainy days there is at least a temporary interruption of solar radiation, some degree of connection between precipitation and monthly mean temperature might be anticipated.

TABLE VI

COEFFICIENTS OF INTER-ANNUAL CORRELATION BETWEEN PRECIPITATION (INCHES OF RAIN) AND MEAN TEMPERATURE (°F.), BY MONTHS, AT METEOROLOGICAL STATIONS IN CENTRAL AND SOUTHERN ALBERTA AND SASKATCHEWAN, 1893-1933

Station	April	May	June	July	August
Edmonton	0.02	0.03	-0.05	-0.42**	-0.31
Calgary	- .20	- .41*	- .49**	- .40*	- .52**
Battleford	- .12	.06	- .15	- .38*	- .40*
Swift Current	- .10	- .32*	- .15	- .37*	- .37*

*Exceeds 5% point (0.31).

**Exceeds 1% point (0.40).

The results in Table VI are as a whole consistent with this supposition. Seventeen of the 20 coefficients listed are negative, indicating that increased precipitation tended to be associated with a lower monthly mean temperature, and the three positive values are all insignificant, the largest being only 0.06. The degree of correlation indicated is however at best only moderate, and is further characterized by a marked seasonal incidence. Considered individually, the coefficients for April at all four stations are insignificant. Two of those for May, and one for June, exceed the 5% point. On the other hand, all of those for July and August may be regarded as significant, and four of the eight attain or exceed the 1% point.

Characteristics of Daily Observations

At the time that this study was begun, records of the daily observations at the four foregoing stations for the years 1916 to 1933 inclusive were available in published form (7). These were examined as described below.

INTRA- AND INTER-ANNUAL VARIATION OF DAILY OBSERVATIONS BY MONTHS

The analysis of variance procedure (2) was applied to the observations recorded for each station. In this way the total daily variance of the mean, maximum, minimum and range in temperature of each month during the 18 years was subdivided as shown in Tables VII to X into one portion arising from differences in the averages of the same month (*e.g.*, April) in different years, and another portion due to daily deviations from the average of the individual months within years. Each of the four tables in question is thus deduced from a total of 11,016 daily observations.

In each table the observations for all five months at all four stations have yielded greater mean squares between years than within years, and with but one exception these differences in variance are individually significant. It would seem, therefore, that the average maximum, minimum, mean and range of the same month in different years are determined in part by the operation of factors additional to those causing variation from day to day within months. The mean squares within years provide a direct measure of the variation of the four temperature characteristics from day to day, based on 18 years' experience at each station. To facilitate comparisons, the corresponding standard deviations are brought together in Table XI.

Although the standard deviations of daily maxima and minima are in general slightly higher for the southern than for the central stations, and for the Saskatchewan than the Alberta ones, there are no pronounced differences between stations in respect of the intra-monthly variation of any of the four series of daily observations. Within stations, on the other hand, the daily maximum temperatures recorded for all five months are seen to have been more variable than the corresponding daily minima. The intra-monthly daily variation of both these quantities, as also that of the mean and range, shows an appreciable seasonal variation. The variability of maximum, minimum and mean is highest in April and lowest in July and August. In the case of the daily range, however, the lowest variability is attained in June and July. Furthermore, the differences between months in the standard deviation of daily range are definitely smaller than the corresponding differences in the standard deviation of daily maximum, minimum or mean. This is indicative of a closer correlation between the maximum and minimum temperature of the same day in the earlier part of the season.

INTRA- AND INTER-ANNUAL CORRELATION BETWEEN DAILY MAXIMA AND MINIMA

The values of the correlation coefficient r , specifying the degree of association between daily maximum and minimum temperature by months both within and between years, were readily deduced from the mean squares given in Tables VIII, IX and X. Denoting the daily maximum temperature by t_1 , the daily minimum by t_2 , the daily range by $t_1 - t_2$, and any mean square

TABLE VII
ANALYSIS OF VARIANCE OF MEAN DAILY TEMPERATURE (°F.), BY MONTHS, 1916-1933

Station	Variance	April		May		June		July		August	
		D.F.	Mean sq.	D.F.	Mean sq.	D.F.	Mean sq.	D.F.	Mean sq.	D.F.	Mean sq.
Edmonton	Between years	17	586.4**	17	173.4**	17	70.1*	17	57.8*	17	141.4**
	Within years	522	87.0	540	52.6	522	39.7	540	30.7	540	29.7
Calgary	Between years	17	581.1**	17	211.0**	17	110.4**	17	64.2**	17	119.0**
	Within years	522	77.7	540	53.3	522	40.2	540	30.2	540	32.6
Battleford	Between years	17	680.9**	17	257.5**	17	164.9**	17	112.7**	17	117.8**
	Within years	522	94.6	540	63.1	522	42.6	540	30.4	540	34.5
Swift Current	Between years	17	523.7**	17	322.9**	17	330.8**	17	84.5**	17	188.1**
	Within years	522	91.6	540	69.7	522	51.0	540	40.2	540	39.0

* Exceeds mean square within years, 5% level of significance.

** Exceeds mean square within years, 1% level of significance.

TABLE VIII
ANALYSIS OF VARIANCE OF MAXIMUM DAILY TEMPERATURE (°F.), BY MONTHS, 1916-1933

Station	Variance	April		May		June		July		August	
		D.F.	Mean sq.	D.F.	Mean sq.	D.F.	Mean sq.	D.F.	Mean sq.	D.F.	Mean sq.
Edmonton	Between years	17	1055.8**	17	353.1**	17	140.6**	17	166.9**	17	257.2**
	Within years	522	130.7	540	92.9	522	66.2	540	55.8	540	60.2
Calgary	Between years	17	970.8**	17	619.1**	17	357.9**	17	308.4**	17	280.5**
	Within years	522	144.6	540	97.8	522	80.0	540	59.8	540	73.1
Battleford	Between years	17	1318.9**	17	450.3**	17	269.2**	17	125.9**	17	217.4**
	Within years	522	137.2	540	102.2	522	73.1	540	54.3	540	66.0
Swift Current	Between years	17	915.3**	17	684.2**	17	555.6**	17	253.2**	17	323.6**
	Within years	522	149.6	540	110.2	522	86.0	540	67.4	540	72.2

* Exceeds mean square within years, 5% level of significance.

** Exceeds mean square within years, 1% level of significance.

TABLE IX
ANALYSIS OF VARIANCE OF MINIMUM DAILY TEMPERATURE, (°F.), BY MONTHS, 1916-1933

Station	Variance	April		May		June		July		August	
		D.F.	Mean sq.	D.F.	Mean sq.	D.F.	Mean sq.	D.F.	Mean sq.	D.F.	Mean sq.
Edmonton	Between years	17	349.7**	17	97.6**	17	93.7**	17	47.6*	17	103.8**
	Within years	522	73.7	540	45.9	522	40.8	540	29.4	540	31.4
Calgary	Between years	17	430.2**	17	79.1**	17	99.5**	17	45.4	17	100.8**
	Within years	522	62.5	540	38.8	522	29.5	540	28.5	540	28.2
Battleford	Between years	17	388.4**	17	236.1**	17	194.7**	17	105.8**	17	105.7**
	Within years	522	89.2	540	60.3	522	42.6	540	31.8	540	37.2
Swift Current	Between years	17	338.8**	17	258.7**	17	245.4**	17	72.1*	17	148.0**
	Within years	522	72.7	540	65.1	522	47.1	540	41.9	540	39.7

*Exceeds mean square within years, 5% level of significance.

**Exceeds mean square within years, 1% level of significance.

TABLE X
ANALYSIS OF VARIANCE OF DAILY RANGE IN TEMPERATURE (°F.), BY MONTHS, 1916-1933

Station	Variance	April		May		June		July		August	
		D.F.	Mean sq.	D.F.	Mean sq.	D.F.	Mean sq.	D.F.	Mean sq.	D.F.	Mean sq.
Edmonton	Between years	17	297.5**	17	212.7**	17	168.9**	17	191.0**	17	158.1**
	Within years	522	67.5	540	68.9	522	56.2	540	48.9	540	63.7
Calgary	Between years	17	585.9**	17	493.0**	17	463.5**	17	450.1**	17	290.7**
	Within years	522	91.6	540	78.0	522	61.0	540	56.7	540	73.2
Battleford	Between years	17	485.9**	17	275.8**	17	273.5**	17	304.5**	17	186.1**
	Within years	522	72.0	540	70.2	522	62.2	540	52.0	540	70.1
Swift Current	Between years	17	417.6**	17	563.5**	17	259.3**	17	326.1**	17	189.8**
	Within years	522	81.0	540	74.3	522	61.4	540	58.2	540	68.9

*Exceeds mean square within years, 5% level of significance.

**Exceeds mean square within years, 1% level of significance.

TABLE XI
INTRA-MONTHLY STANDARD DEVIATION (°F.) OF DAILY MEAN, MAXIMUM, MINIMUM, AND RANGE IN TEMPERATURE, 1916-1933

Month	Edmonton				Calgary				Battleford				Swift Current			
	Mean	Max.	Min.	Range	Mean	Max.	Min.	Range	Mean	Max.	Min.	Range	Mean	Max.	Min.	Range
April	9.3	11.4	8.6	8.2	8.8	12.0	7.9	9.6	9.7	11.7	9.4	8.5	9.6	12.2	8.5	9.0
May	7.3	9.6	6.8	8.2	7.3	9.9	6.2	8.8	8.0	10.1	7.8	8.4	8.4	10.5	8.1	8.6
June	6.2	8.1	6.4	7.5	6.3	8.9	5.4	7.8	6.5	8.6	6.5	7.9	7.1	9.3	6.9	7.8
July	5.5	7.5	5.4	7.0	5.5	7.7	5.3	7.5	5.5	7.4	5.6	7.2	6.3	8.2	6.5	7.6
August	5.4	7.8	5.6	8.0	5.7	8.6	5.3	8.6	5.9	8.1	6.1	8.4	6.2	8.5	6.3	8.3

by s^2 , it is well known that

$$s_{t_1-t_2}^2 = s_{t_1}^2 + s_{t_2}^2 - 2r_{t_1 t_2} \cdot s_{t_1} s_{t_2}$$

whence

$$r_{t_1 t_2} = \frac{s_{t_1}^2 + s_{t_2}^2 - s_{t_1-t_2}^2}{2s_{t_1} s_{t_2}}.$$

Substituting the appropriate mean squares in the foregoing expression, the values of r shown in Tables XII and XIII were obtained.

Table XII shows for each of the five months the intra-monthly correlation coefficients computed from the mean squares within years. All of these are statistically significant, the 1% point for the shorter months (30 days per

TABLE XII

INTRA-MONTHLY CORRELATION COEFFICIENTS (r) BETWEEN DAILY MAXIMUM AND MINIMUM TEMPERATURE ($^{\circ}\text{F.}$)

Station	April	May	June	July	August
Edmonton	0.70	0.50	0.49	0.45	0.32
Calgary	.61	.48	.50	.38	.31
Battleford	.70	.59	.48	.41	.33
Swift Current	.68	.60	.56	.49	.40

Value of r at 1% point ≤ 0.11

annum, 521 degrees of freedom) being $r = 0.11$. There is therefore a definite tendency within each month during the period considered for above-average daily maximum temperatures to be associated with above-average minima, and below-average maxima with below-average minima. This tendency is however more pronounced in spring than in summer. At all four stations the correlation coefficients decline progressively from April to August, the degree of association indicated in the latter month being very moderate.

The coefficients obtained from the mean squares between years are shown in Table XIII. These indicate the degree of association between the mean daily maximum and mean daily minimum temperature of the specified months from year to year over the period 1916-1933. As the mean squares in this instance are each derived from only 17 degrees of freedom, the resulting

TABLE XIII

INTER-ANNUAL CORRELATION COEFFICIENTS (r) BETWEEN MONTHLY MEAN MAXIMUM AND MINIMUM TEMPERATURES ($^{\circ}\text{F.}$), 1916-1933

Station	April	May	June	July	August
Edmonton	0.91**	0.64**	0.28	0.13	0.62**
Calgary	.63**	.46	— .02	— .41	.27
Battleford	.85**	.63**	.42	— .32	.45
Swift Current	.75**	.45	.73**	.00	.64**

Value of r at 5% point = 0.47.
Value of r at 1% point = 0.59.

**Exceeds 5% point.*
***Exceeds 1% point.*

coefficients are determined with a much lower precision than those of the preceding Table XI, if considered as estimates of the true values characteristic of the climate of the stations rather than as merely descriptive statistics of the particular 18 seasons examined. Even so, there is evidence of a rather pronounced seasonal variation in the inter-annual correlation. The association would seem to be close in April, when the variation in the temperature conditions from year to year may be considerable (Table II); to decline to a minimum in June and July, in which months inter-annual variation is smaller; and to increase again in August. Thus warmer-than-average Aprils tend to be characterized by mean maxima and minima which are both above-average. In mid-summer, on the contrary, this tendency no longer prevails.

In this connection, however, it should be pointed out that during the spring and summer months the minimum temperature recorded for any particular day usually occurs during the early morning hours, with the result that the foregoing correlations are, in effect, between minimum and succeeding maximum rather than between maximum and succeeding minimum. Additional calculations were therefore made in order to determine the latter for two of the four stations, namely Edmonton and Swift Current. The results are given in Tables XIV, XV and XVI.

TABLE XIV
INTER-ANNUAL COEFFICIENTS OF CORRELATION (r) BETWEEN MONTHLY MEAN MAXIMUM AND SUCCEEDING MINIMUM TEMPERATURES ($^{\circ}\text{F.}$), 1916-1933

Station	April	May	June	July	August
Edmonton	0.91**	0.61**	0.28	0.17	0.65**
Swift Current	.62**	.44	.74**	— .02	.65**

Value of r at 5% point = 0.47.

Value of r at 1% point = 0.59.

*Exceeds 5% point.

**Exceeds 1% point.

TABLE XV
INTRA-MONTHLY COEFFICIENTS OF CORRELATION (r) BETWEEN DAILY MAXIMUM AND SUCCEEDING MINIMUM TEMPERATURES ($^{\circ}\text{F.}$), 1916-1933

Station	April	May	June	July	August
Edmonton	0.74	0.68	0.60	0.60	0.54
Swift Current	.72	.66	.64	.58	.58

Value of r at 1% point ≤ 0.11 .

TABLE XVI
COEFFICIENTS OF REGRESSION (b) OF SUCCEEDING MINIMUM ON DAILY MAXIMUM TEMPERATURE, ($^{\circ}\text{F.}$), 1916-1933

Station	April	May	June	July	August
Edmonton	0.55 ± 0.02	0.48 ± 0.02	0.46 ± 0.03	0.44 ± 0.02	0.40 ± 0.03
Swift Current	$.50 \pm .02$	$.51 \pm .02$	$.47 \pm .02$	$.47 \pm .03$	$.43 \pm .03$

As might be expected, the correlation between mean monthly maximum and minimum determined from the 17 degrees of freedom between years is not significantly affected, and the remarks made with reference to the preceding Table XIII apply also to the inter-annual coefficients in Table XIV.

The intra-monthly correlations, based on 521 residual degrees of freedom for April and June, and 539 for May, July and August, are given in Table XV. All again exceed the 1% point, and, as expected, the coefficients in this table are uniformly higher than the corresponding ones in Table XI. As before, however, the degree of association indicated is highest for the spring months, and only moderate for the summer.

Table XVI contains the intra-monthly regression coefficients of minimum temperature on preceding daily maximum for both Edmonton and Swift Current. These range from 0.40 to 0.55° F., indicating that the amplitude of associated variations in the minima was on the average about half that of the fluctuations in daily maxima. Within any month, the regression coefficients for the two stations do not differ significantly, but at both places they are somewhat higher for spring than for summer. Thus at Edmonton, a 10-degree difference in daily maxima during April resulted on the average in a difference of 5.5° in the subsequent minima, whereas in August the corresponding average difference in the minima was only 4.0°.

FREQUENCY DISTRIBUTION OF DAILY OBSERVATIONS

The frequency distribution between 4° F. class intervals of the daily maximum and minimum readings for each month at the four stations during the 18-year period is represented by means of histograms in Fig. 6. Table XVII lists the numerical values of certain statistics descriptive of the individual distributions, computed in each case from the ungrouped data.

Five statistics are given in each case. The first of these is simply the arithmetic mean of the aggregate of observations, 540 or 558 in number, depending on whether the month in question comprises 30 or 31 days. Next follow the second (m_2), third (m_3) and fourth (m_4) moments about the mean, *i.e.*, the mean square, mean cube and mean fourth power of the 540 or 558 deviations from the arithmetic mean. The mean square deviation or variance (m_2) is indicative of the extent of dispersion in the distribution, whilst the

pure numbers $\sqrt{B_1} = \frac{m_3}{m_2^{3/2}}$ and $B_2 = \frac{m_4}{m_2^2}$, also given in Table XVII, permit

comparisons to be made between the symmetry and modality (*i.e.*, the tendency of the observations to cluster numerously about some modal value) of distributions of different absolute dispersion. If the observations follow exactly the "Normal" law of frequency $\sqrt{B_1} = 0$ and $B_2 = 3$. Tables have been published by E. S. Pearson (8) enabling the significance of observed deviations from these values, in samples of different sizes, to be tested.

In addition to the average seasonal trend of the maximum and minimum, there may also be observed from the two columns of means in Table XVII a

further effect attributable to the interacting differences in latitude and elevation between Edmonton and Calgary. The mean minimum temperature for each of the five months is lower for Calgary than for Edmonton, whereas the mean maximum is higher, except for May. On the other hand, there is little difference between the mean minima for Battleford and Swift Current, excepting those for April, when the value for the southern station is the higher. The five mean maxima are again consistently higher for Swift Current than for Battleford.

The coefficients $\sqrt{B_1}$ and B_2 exhibit certain trends over the five-month period. In the case of the daily minima, $\sqrt{B_1}$ is significantly negative at all four stations for April. The asymmetry of the four distributions is evident

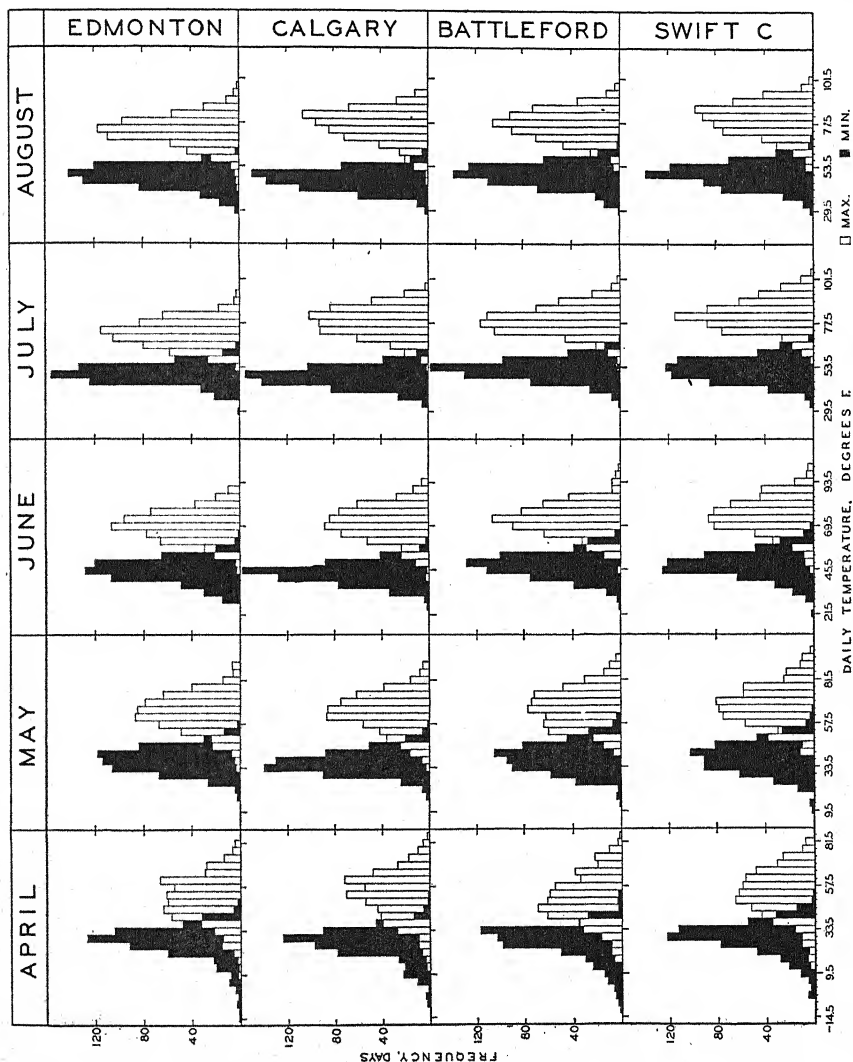


FIG. 6. Frequency distribution of daily maximum (open columns) and minimum (solid columns) temperatures at Edmonton (central Alberta), Calgary (southern Alberta), Battleford (central Saskatchewan) and Swift Current (southern Saskatchewan), 1910-1933.

TABLE XVII
STATISTICS OF FREQUENCY DISTRIBUTION OF DAILY MAXIMUM AND MINIMUM AIR TEMPERATURES (°F.) AT METEOROLOGICAL STATIONS
IN CENTRAL AND SOUTHERN ALBERTA AND SASKATCHEWAN, 1916-1933

Month	Daily minima						Daily maxima					
	Mean	Second moment (ms)	Third moment (ms)	Fourth moment (ms)	$\sqrt{B_1}$	B_2	Mean	Second moment (ms)	Third moment (ms)	Fourth moment (ms)	$\sqrt{B_1}$	B_2
April—												
Edmonton	27.9	82.25	-819.70	36,934.63	-1.099**	5.460**	50.8	159.59	-493.83	75,301.11	-0.245*	2.957
Calgary	26.5	73.99	-461.02	22,229.79	-0.724**	4.061**	52.4	170.30	-590.33	71,421.32	-0.266**	2.463**
Battleford	26.9	98.43	-865.79	38,235.01	-0.887**	3.946**	50.2	174.14	6.88	82,000.57	0.003	2.704
Swift Current	28.7	80.91	-528.40	27,703.58	-0.726**	4.232**	52.6	173.44	-650.84	88,272.71	-0.171	2.934
May—												
Edmonton	38.0	47.41	54.40	5,993.49	0.167	2.667	63.7	100.62	-20.66	30,289.25	-0.020	2.902
Calgary	36.3	40.00	65.31	5,116.61	0.258**	3.198	63.3	113.51	-297.89	43,763.00	-0.239**	3.397
Battleford	38.9	65.58	29.15	12,027.78	0.055	2.797	65.8	114.14	111.60	32,070.85	0.092	2.462**
Swift Current	38.5	70.87	-17.40	15,158.65	-0.029	3.018	66.6	127.53	115.68	44,984.99	0.080	2.766
June—												
Edmonton	45.2	42.35	-45.13	5,180.43	-0.164	2.888	70.0	68.38	-46.54	12,874.62	-0.092	2.753
Calgary	43.9	31.66	0.89	2,829.88	0.005	2.823	70.8	89.57	-197.36	23,388.81	-0.233*	2.915
Battleford	48.1	47.30	-31.80	6,534.57	-0.098	2.921	72.8	79.17	-39.83	19,823.98	-0.057	3.163
Swift Current	48.2	53.25	-36.45	8,959.74	-0.094	3.160	75.0	100.66	-74.54	29,970.64	-0.074	2.958
July—												
Edmonton	49.5	29.88	-13.20	2,561.07	-0.081	2.869	75.0	59.13	-56.75	9,989.20	-0.125	2.857
Calgary	48.0	28.92	-9.48	2,245.59	-0.061	2.685*	77.8	67.31	-34.30	12,116.45	-0.062	2.67*
Battleford	52.1	34.00	-21.11	3,401.38	-0.107	2.951	79.2	56.40	32.51	9,647.67	0.078	3.033
Swift Current	52.4	42.72	2.52	4,816.91	0.009	2.639*	81.9	72.91	10.81	12,913.68	0.017	2.813
August—												
Edmonton	47.5	33.55	-77.16	3,184.45	-0.397**	2.829	72.4	66.06	-144.70	14,622.47	-0.270**	3.351*
Calgary	46.1	30.40	-7.92	2,426.02	-0.047	2.625*	75.5	79.30	-344.55	18,098.73	-0.488**	2.878
Battleford	49.1	39.20	-51.40	4,496.04	-0.209*	2.926	76.6	70.47	-88.11	13,711.58	-0.149	2.761
Swift Current	49.4	42.94	-43.21	4,960.57	-0.154	2.690*	79.8	79.73	-239.14	17,944.52	-0.336**	2.823

*Deviation of $\sqrt{B_1}$ from zero, or of B_2 from 3,000, exceeds 5% point.

**Deviation of $\sqrt{B_1}$ from zero, or of B_2 from 3,000, exceeds 1% point.

from Fig. 6, the mode being in excess of the mean, and moderately above- and markedly below-average daily minima being more numerous than moderately below- and markedly above-average ones respectively. The high values of B_2 are indicative of a further feature, namely the pronounced modality of the daily minima for this month. Thus at Edmonton the total range of variation by four-degree class intervals is from -16.5° to $+51.5^\circ$ F. or 68° ; but the 12-degree interval 23.5° to 35.5° comprises 323 or 60% of the total of 540 daily values. The same 12-degree interval was found to include at Calgary 311 or 58%, at Battleford 319 or 59%, and at Swift Current 312 or 58% of the daily observations. The April daily maxima also show tendencies in the direction of a negatively skewed frequency distribution at three of the four stations, but the values of $\sqrt{B_1}$ indicate that this symptom is relatively less marked than in the case of the daily minima. Furthermore, there is not so pronounced a concentration of the observations within the modal region, all four values of B_2 being below rather than above the Normal value of 3.000, although the deviation from normality is statistically significant in only one instance.

In the succeeding months of May, June and July the variance of both maximum and minimum diminishes progressively; also, the distribution of the daily observations approaches more closely the Normal form, corresponding deviations above and below the mean tending to occur with equal frequency. The August observations on the other hand show a reversion to the negatively skewed distribution of both daily maxima and minima which is characteristic of those for April. In August however the asymmetry is more pronounced in the maxima than in the minima, and the concentration of frequency in the modal region is in general in defect rather than in excess of the Normal expectation. When examined in this way, therefore, each of the five months is seen to have individual temperature characteristics which, for the 18-year period considered, show a generally similar seasonal progression at the stations in all four districts.

Table XVIII lists two temperature attributes of some agricultural interest, namely the percentage of the daily minima falling below 40° F. and the percentage of the daily maxima attaining or exceeding 80° F., and permits comparisons to be made both between months and between stations.

TABLE XVIII

PERCENTAGE OF DAILY MINIMUM AIR TEMPERATURES BELOW 40° F. AND OF DAILY MAXIMA OF 80° F. OR ABOVE, AT METEOROLOGICAL STATIONS IN CENTRAL AND SOUTHERN ALBERTA AND SASKATCHEWAN BY MONTHS, 1916-1933

Month	Minima below 40° F., %				Maxima 80° F. or above, %			
	Edmonton	Calgary	Battleford	Swift Current	Edmonton	Calgary	Battleford	Swift Current
April	93	96	93	91	1	1	1	1
May	58	70	52	53	4	5	10	12
June	17	21	10	10	12	20	23	33
July	4	5	1	1	29	46	47	62
August	9	13	6	6	18	38	38	55

The frequency distribution of the daily mean temperatures (average of maximum and minimum) was also determined for each month and station, and is shown graphically in Fig. 7. The individual daily maxima and minima being positively correlated, the frequency distributions derived from them show seasonal trends in variance, skewness and kurtosis similar to those illustrated in Fig. 6.

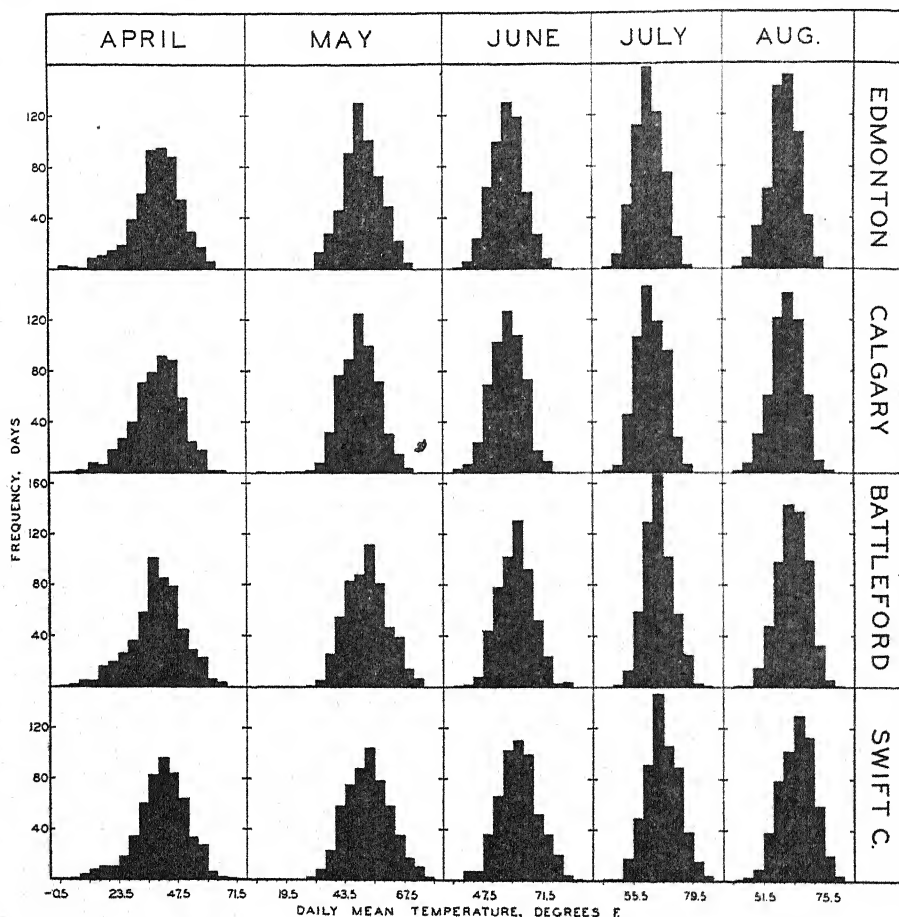


TABLE XIX

PERCENTAGE FREQUENCY DISTRIBUTION AND MEAN VALUE OF DAILY RANGE IN AIR TEMPERATURE (°F.) BY MONTHS AT EDMONTON, CALGARY, BATTLEFORD AND SWIFT CURRENT, 1916-1933

Class interval	April	May	June	July	August
0 to 7° incl.	3	2	2	1	1
8 to 11	7	4	3	2	3
12 to 15	11	6	7	5	4
16 to 19	12	10	9	8	8
20 to 23	15	13	17	13	13
24 to 27	15	15	18	17	16
28 to 31	14	16	18	20	17
32 to 35	12	17	14	18	18
36 to 39	7	11	7	10	11
40 to 43	3	4	3	5	6
44 -	2	2	1	2	3
Mean daily range, °F.	24.0	26.9	25.8	28.0	28.0

upward as the season advances, this being accompanied by a decrease in the proportion of smaller daily ranges and an increase in that of the larger ones. It may be noted that the observations for June show a recession from this trend; this is presumably connected with the fact that June is the month of maximum average precipitation in the Plains area. Seasonal change in the average daily range is however much less pronounced than that in the temperature itself. The mean daily temperature at the four stations for 1916-1933 increases from 39.5° F. in April to 64.0° F. in July, but the corresponding variation in the mean daily range is only from 24.0° to 28.0°.

Separate tabulations showed a similar seasonal trend to be characteristic of the observations of daily range at each of the four stations individually, but application of the χ^2 test of homogeneity to the separate tabulations indicated statistically significant differences between stations for each of the five months. These seemed to be attributable mainly to the fact that the mean daily range for each month was higher at Calgary and Swift Current than at Edmonton and Battleford.

Hourly Temperatures at Swift Current

For some years it was the custom of the Meteorological Service of Canada to publish in full the hourly temperatures recorded at a number of stations by automatic instruments. Of the four stations here considered, Swift Current is the only one for which such results were thus available.

DIURNAL VARIATION

The hourly observations at this station during April, May, June, July and August of the years 1922, 1923, 1925 and 1927 were used to compute the average diurnal variation in temperature for each month, shown in Fig. 8 and Table XX. Had the records been complete, each hourly point in the curves would have been the average of 120 daily observations in April and

June, and of 124 in May, July and August. Actually, however, there were some days on which the instrument was temporarily out of order. Whenever this occurred, the observations for the entire day concerned were excluded from the averages.

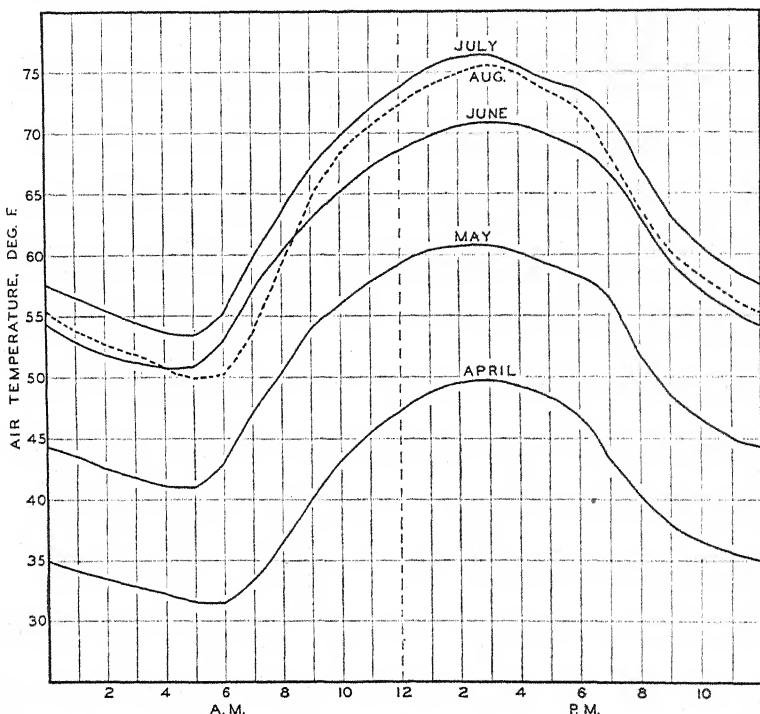


FIG. 8. Average diurnal temperature curves for five spring and summer months at Swift Current, Saskatchewan. Computed from hourly observations recorded in 1922, 1923, 1925 and 1927.

In spite of the differences in respect of the general temperature level, the average hourly value attains its maximum at this station at 3 p.m. in all five months. The subsequent decline is seen to be most rapid in the earlier part of the night. By 10 or 11 p.m. a more moderate rate of cooling supervenes, which is maintained until arrested next morning by renewed insolation. The hour of minimum average temperature, being influenced by the time of sunrise, varies from 6 a.m. in April to 4 a.m. in June.

All five diurnal curves are to some extent asymmetrical, the number of hourly intervals with falling temperature being in excess of the number with rising temperature. On the other hand, the average rate of cooling is in consequence slower than the average rate of heating. As is indicated in Table XX, this disproportion is least in evidence in the observations for June, the month of longest day, and most pronounced in those for April.

TABLE XX
HOURLY TEMPERATURE (°F.) AT SWIFT CURRENT, SASKATCHEWAN, BY MONTHS.
AVERAGE OF FOUR YEARS' OBSERVATIONS

Hour	April	May	June	July	August
1 a.m.	34.2	43.6	52.8	56.4	53.8
2	33.4	42.6	51.8	55.5	52.7
3	32.8	41.8	51.2	54.5	51.9
4	32.2	41.1	50.7	53.7	50.8
5	31.6	41.0	50.8	53.4	50.0
6	31.5	43.0	53.0	55.2	50.2
7	33.4	47.1	56.7	59.6	53.5
8	36.5	50.5	60.2	63.6	59.2
9	40.1	54.0	63.2	67.2	64.7
10	43.2	56.0	65.2	69.8	68.4
11	45.6	57.8	67.2	72.0	70.5
12	47.3	59.3	68.6	73.7	72.3
1 p.m.	48.8	60.4	69.8	75.4	73.9
2	49.5	60.7	70.6	76.2	74.9
3	49.8	60.8	70.8	76.5	75.6
4	49.3	60.2	70.7	75.6	74.9
5	48.4	59.2	69.8	74.4	73.5
6	46.8	58.2	68.8	73.6	72.0
7	43.4	56.6	66.8	71.5	68.2
8	40.3	51.8	63.2	67.3	63.7
9	38.0	48.6	59.4	63.2	60.2
10	36.6	46.6	57.2	60.7	58.3
11	35.6	45.0	55.4	58.8	56.4
12	34.9	44.2	54.2	57.5	55.2
No. hourly intervals with rising temp.	9	10	11	10	9
No. hourly intervals with falling temp.	15	14	13	14	15
Average hourly rise	2.0°	2.0°	1.8°	2.3°	2.8°
Average hourly fall	1.2°	1.4°	1.5°	1.6°	1.7°

FREQUENCY DISTRIBUTION OF HOURLY TEMPERATURES

Fig. 9 shows diagrammatically the percentage frequency distribution, between 4° F. class intervals, of the aggregate of hourly temperatures recorded during each of the five months of the four years considered. The full complement of observations for each distribution is 720 in the case of April and June, and 744 in the case of May, July and August, but as before, whenever the instrument was temporarily out of order, the records for the entire day concerned were excluded.

The general seasonal trend of temperature, reaching its maximum in July, is of course well in evidence, but it is also clear that in the spring months (April and May) there may be an appreciable annual variation in the aggregate of hourly values. The other months studied show on the whole more consistency over the four years.

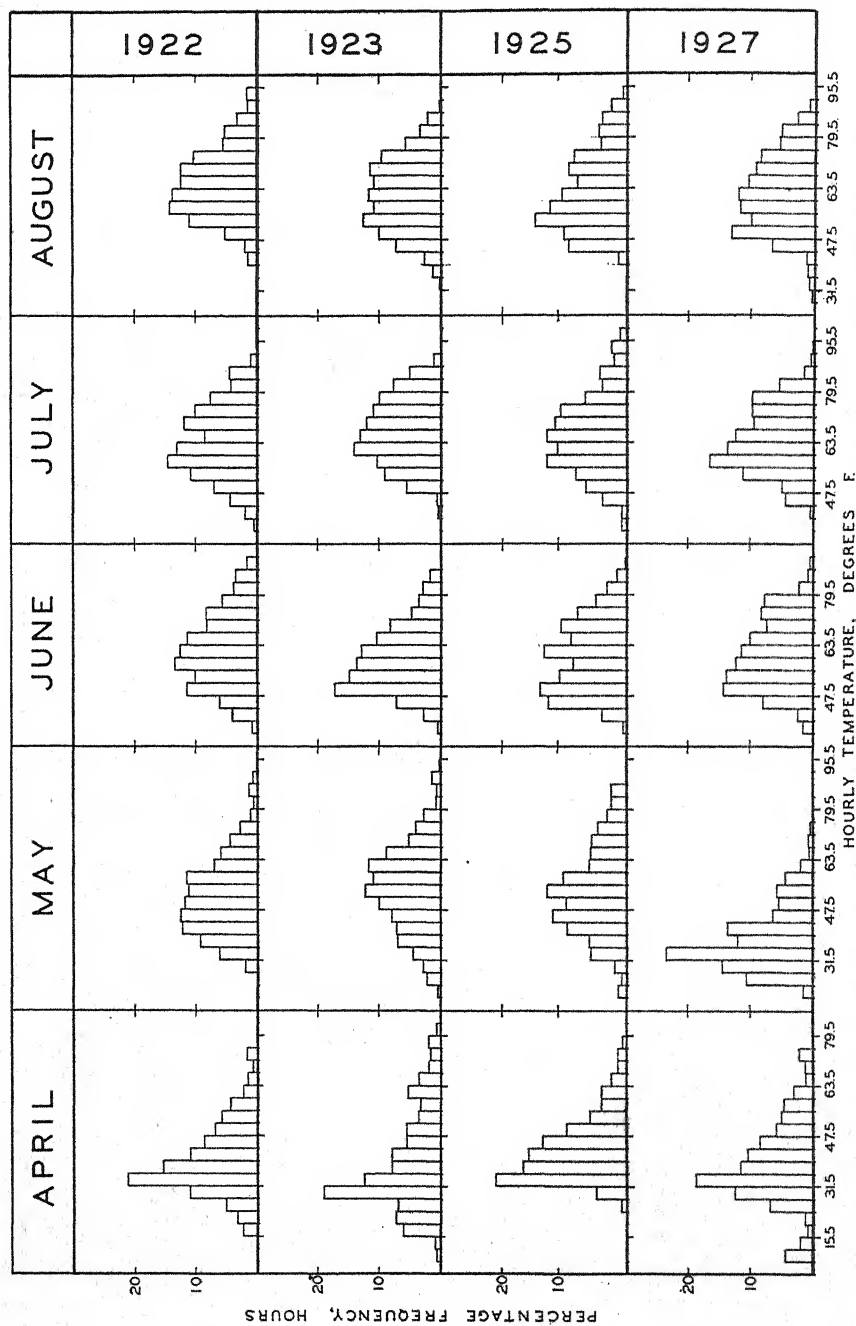


FIG. 9. Percentage frequency distribution of hourly temperatures at Swift Current, Saskatchewan, during five spring and summer months of 1922, 1923, 1925, and 1927.

All of the 20 monthly frequency distributions deviate more or less from symmetry, hours with below-average temperatures being in excess of those with above-average ones. The unequal concentration of the hourly values is emphasized in Table XXI, which shows the four quartile ranges, *i.e.*, the

TABLE XXI
QUARTILE RANGES (°F.) OF FREQUENCY DISTRIBUTION OF HOURLY TEMPERATURE OBSERVATIONS
AT SWIFT CURRENT

Month	Year	Q ₁ (0-25%)	Q ₂ (25-50%)	Q ₃ (50-75%)	Q ₄ (75-100%)
April	1922	15.5-32.4	32.4-37.1	37.1-46.3	46.3-74.5
	23	7.5-29.4	29.4-34.7	34.7-48.5	48.5-81.5
	25	24.5-34.7	34.7-41.3	41.3-48.7	48.7-78.5
	27	7.5-30.6	30.6-36.2	36.2-46.3	46.3-76.5
	4 yr. average quartile range	18.0	5.6	10.1	30.3
May	1922	30.5-42.3	42.3-50.0	50.0-59.0	59.0-88.5
	23	21.5-44.7	44.7-54.4	54.4-62.9	62.9-92.5
	25	20.5-43.5	43.5-52.6	52.6-62.8	62.8-87.5
	27	28.5-39.2	39.2-43.5	43.5-51.4	51.4-81.5
	4-yr. average quartile range	17.2	7.7	8.9	28.5
June	1922	36.5-52.5	52.5-60.5	60.5-71.1	71.1-91.5
	23	41.5-55.0	55.0-62.0	62.0-69.8	69.8-90.5
	25	38.5-49.6	49.6-59.6	59.6-68.7	68.7-88.5
	27	35.5-51.3	51.3-58.7	58.7-68.1	68.1-89.5
	4-yr. average quartile range	14.1	8.1	9.2	20.6
July	1922	37.5-55.6	55.6-62.7	62.7-72.4	72.4-91.5
	23	39.5-59.1	59.1-66.5	66.5-74.9	74.9-91.5
	25	38.5-57.2	57.2-65.0	65.0-73.8	73.8-98.5
	27	40.5-56.6	56.6-63.1	63.1-72.3	72.3-92.5
	4-yr. average quartile range	18.1	7.2	9.0	20.1
August	1922	39.5-57.2	57.2-64.2	64.2-72.5	72.5-94.5
	23	34.5-52.7	52.7-60.8	60.8-70.1	70.1-88.5
	25	42.5-52.3	52.3-60.1	60.1-71.2	71.2-94.5
	27	30.5-52.3	52.3-60.7	60.7-70.6	70.6-90.5
	4-yr. average quartile range	16.9	7.8	9.6	20.9

temperature intervals comprising 0-25%, 25-50%, 50-75% and 75-100% of the hourly observations for each month each year, and also the average range of each quartile for the four years. This inequality is, like most of the other temperature characteristics dealt with, of a seasonal nature, being most marked in the spring and least pronounced in the summer months. Thus in April the four-year average range below and above the 50% point is 23.6 and 40.4° F. respectively, whereas for July the corresponding figures are 25.3 and 29.1°.

COMPARISON OF 2- AND 24-POINT DAILY MEANS

The hourly records may be further utilized to compare the daily mean temperature, computed in the customary way by averaging the maximum and minimum, with that obtained by averaging the 24 hourly values. In view of the asymmetry of the average diurnal curves, it might be expected that the two means would not in general coincide, and the mean of the daily maximum and minimum (m_2) was in fact found to be on the average in excess of the mean of the 24 hourly observations (m_{24}). The magnitude of the average discrepancy seems to be a function of the season, varying as shown in Table XXII from 1.47° F. in April to a minimum of 0.74° F. in July, in the case of the four-year period studied.

TABLE XXII

COMPARISON OF MEAN DAILY MAXIMUM AND MINIMUM TEMPERATURE (m_2) AND MEAN OF 24 HOURLY OBSERVATIONS (m_{24}) AT SWIFT CURRENT, SASKATCHEWAN, BY MONTHS

—	April	May	June	July	August
Average difference, ($m_2 - m_{24}$), °F.	1.47	0.94	0.79	0.74	0.83
S.D. of observed differences, °F.	2.1	3.1	2.9	2.7	2.4
Max. diff. in 4-yr. period, °F.	+7.6	+9.1	+9.7	+9.4	+8.0

Although the average discrepancy between m_2 and m_{24} is thus of moderate dimensions, the deviations actually occurring on individual days vary considerably, as is indicated by the standard deviations given in Table XXII, and are sometimes quite large (see bottom line of Table XXII, and also Fig. 10). The source of such fluctuations is of course to be found in the various departures of the individual daily temperature sequences from the average diurnal trend, occasioned by cloudiness, air movements, precipitation, etc. Numerical specification of such departures, and their relation to the deviations of m_2 from m_{24} , has not been undertaken. It has however been noted that there is some correlation between the daily range in temperature and the magnitude of ($m_2 - m_{24}$), such that as the range increases, the excess of m_2 over m_{24} tends to diminish.

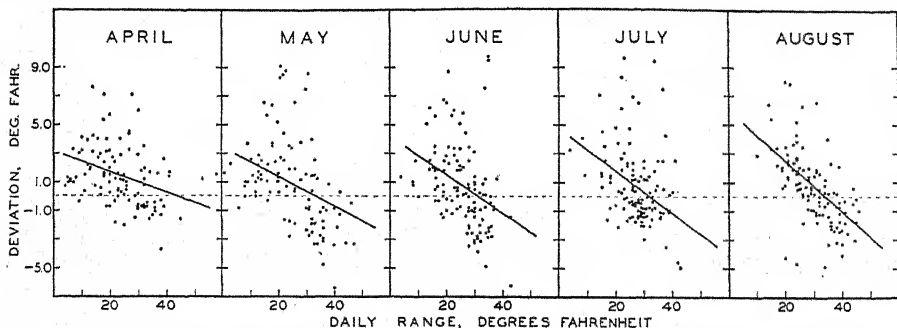


FIG. 10. Relation between deviation of two-point from 24-point daily mean, and daily range in temperature, at Swift Current, Saskatchewan. Computed from four years' data (1922, 1923, 1925 and 1927).

Correlation and regression coefficients, calculated from the four years' data, are shown in Table XXIII. The regression coefficients, specifying the average decrement in ($m_2 - m_{24}$) for each increase of 1° F. in the daily

TABLE XXIII

CORRELATION BETWEEN DAILY RANGE IN TEMPERATURE AND DEVIATION OF MEAN OF DAILY MAXIMUM AND MINIMUM FROM MEAN OF 24 HOURLY OBSERVATIONS ($m_2 - m_{24}$) AT SWIFT CURRENT, SASKATCHEWAN, BY MONTHS

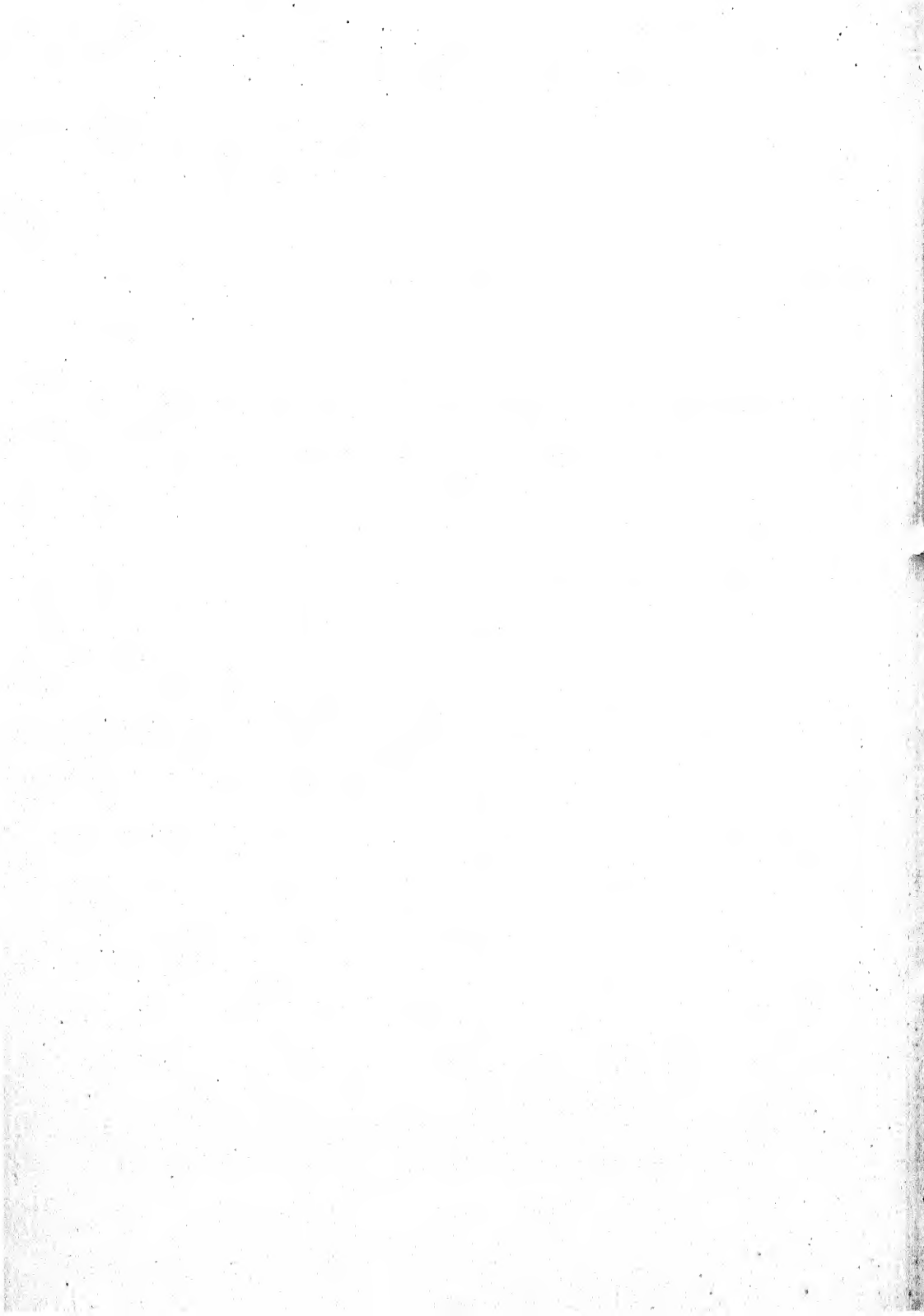
—	April	May	June	July	August
Correlation coefficient*	0.32	0.38	0.36	0.39	0.59
Regression coefficient, °F.	-.07	-.10	-.13	-.14	-.18
Residual S.D., °F.	2.0	2.8	2.7	2.5	1.9

*1% point ≤ 0.25 .

range, increase progressively in absolute magnitude from 0.07° F. in April to 0.18° in August. However, although all five correlation coefficients are statistically significant, the degree of association indicated is only moderate and, as is evident from Fig. 10, accounts for only a minor proportion of the observed variance of ($m_2 - m_{24}$). This is natural, since differences in the daily range reflect only imperfectly the many possible variations in the actual daily temperature sequences. In consequence, it would seem that under the conditions prevailing at Swift Current, the agreement between m_2 and m_{24} may be definitely less satisfactory than is suggested by *e.g.*, the comments of the U.S. meteorologist Kincer (5, p. 5).

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FUSARIUM SPP. AS ROOT PARASITES OF ALFALFA AND SWEET CLOVER IN ALBERTA¹

By M. W. CORMACK²

Abstract

Five pathogenic species predominated among the numerous isolates of *Fusarium* obtained from diseased roots of alfalfa and sweet clover in Alberta. Of these, the closely related species *F. avenaceum* (Fr.) Sacc. and *F. arthrosporioides* Sherb. appear most important, because they occur commonly and can cause serious injury to the roots, both in the early spring and during the growing season. *F. culmorum* (W.G.Sm.) Sacc. is very virulent during the summer, but is apparently non-pathogenic in the early spring. At both times *F. Poae* (Peck) Wr. and *F. Scirpi* Lamb. et Fautr. var. *acuminatum* (Ell. et Ev.) Wr. usually behave as weak pathogens. With the exception of *F. avenaceum* on alfalfa and sweet clover, and *F. Scirpi* var. *acuminatum* on alfalfa, these species have not been previously reported as occurring on the host plants indicated.

Cardinal temperatures for growth in pure culture were:— *F. avenaceum* and *F. arthrosporioides*: -2° , 24° , and 34° C.; *F. culmorum*: 3° , 24° to 27° , and 34° to 36° C.; *F. Poae*: -2° , 20° to 24° , and 32° C.; *F. Scirpi* var. *acuminatum*: 1° , 24° , and 34° C. All five species grew well at hydrogen ion concentrations ranging from pH 4.0 to 9.5. Carbon dioxide concentrations up to 20% had very little effect on the growth of *F. avenaceum*, *F. arthrosporioides*, or *F. Poae*, but the higher concentrations retarded the growth of *F. culmorum* and *F. Scirpi* var. *acuminatum*. The retarding effect of carbon dioxide was greater at 5° C. than at room temperature.

F. avenaceum produced more infection at temperatures up to 24° C. than at 27° C. At 27° C., infection was much lighter in dry soil than in moist soil. *F. culmorum* caused severe damage at 18° to 27° C., but did not attack the roots at low temperatures. *F. avenaceum* usually attacked roots of sweet clover more severely than those of alfalfa. All varieties of both hosts tested proved susceptible. In the absence of wounds, *F. avenaceum* readily entered roots through the basal tissues of branch roots, or through lenticels. Variant forms of this pathogen, which occurred frequently in pure culture, proved decidedly less pathogenic than the original isolates.

Alfalfa and sweet clover roots were attacked by an isolate of *F. avenaceum* obtained from diseased roots of *Vicia americana*. *F. avenaceum*, *F. arthrosporioides*, and *F. culmorum* from alfalfa and sweet clover proved pathogenic to roots of *Trifolium* spp. and to seedlings of wheat, oats, and barley. Certain isolates from the cereals were pathogenic to roots of alfalfa and sweet clover, and thus certain limits to crop rotation in reducing the root-rot damage caused by these pathogens are indicated.

During recent years several fungi have been found parasitizing roots of alfalfa and sweet clover in Alberta. *Plenodomus meliloti* Dearness and Sanford (12) and *Cylindrocarpon Ehrenbergi* (5) have proved of primary importance following the winter dormancy period, while *Sclerotinia* sp. (4) has been

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destructive during the growing season, as well as in the early spring. At both times *Fusarium* spp. predominated among the other fungi isolated from diseased roots, and the prevalence of certain species suggested a parasitic relationship. After eliminating the purely saprophytic forms in preliminary pathogenicity tests, a detailed study was made of the pathogenic isolates representing the following species: *F. avenaceum* (Fr.) Sacc., *F. arthrosporioides*, Sherb., *F. culmorum* (W. G. Sm.) Sacc., *F. Poae* (Pk.) Wr., and *F. Scirpi* Lamb. et Fautr. var. *acuminatum* (Ell. et Ev.) Wr. This investigation has been chiefly concerned with the root rots of alfalfa and sweet clover caused by these species, and with the host relations and physiology of the isolates.

Review of Literature

In a recent book by Wollenweber and Reinking (21), 20 distinct species or varieties of *Fusarium* are listed as occurring on alfalfa, and three species are listed for sweet clover. These include *F. avenaceum* on alfalfa and sweet clover, and *F. Scirpi* var. *acuminatum* on alfalfa, but no detailed studies have been reported on root rots caused by these species. The other three species studied herein, namely *F. arthrosporioides*, *F. culmorum*, and *F. Poae* have apparently not been previously reported on alfalfa or sweet clover. However, all five species are widely distributed, and are important parasites of other plants (20).

Root diseases of alfalfa and sweet clover caused by *Fusarium* spp. have received very little detailed attention in the past. The most comprehensive study reported is that of Weimer (17), on the wilt disease of alfalfa in Mississippi caused by *F. oxysporum* var. *Medicaginis*. Other reports are mainly observational and frequently do not include proof of parasitism or identification of the species concerned. Since most of these reports are reviewed by Weimer (17), only those having a bearing on the present problem will be mentioned here.

Fergus and Valteau (7) found that more than 15 distinct species of *Fusarium*, obtained from alfalfa roots and other sources, were highly pathogenic to sterile alfalfa and clover seedlings growing on agar in test tubes. Other workers, using more natural conditions in their infection experiments, have obtained quite different results. Weimer (18) found that *Fusarium* spp. predominated in the tissues of alfalfa roots suffering from winter injury, but concluded, from the results of infection experiments, that these fungi were mainly saprophytes, or weak parasites. Peltier and Tysdal (11) came to a similar conclusion regarding several species of *Fusarium* which they isolated from rotted alfalfa roots. For many years alfalfa root rot attributed to *Fusarium* spp. has been reported from Missouri, but Scott (14) finally decided that invasion of the roots by these fungi under natural conditions was largely secondary, and occurred in wounds caused by cultural practices or winter injury. As far as is known, none of the above-mentioned studies were concerned with the species found most pathogenic in the present investigation.

Prevalence in Alberta

Under natural conditions, root rot of alfalfa and sweet clover caused by *Fusarium* spp. cannot be distinguished with certainty from that produced by other pathogens. However, isolation studies give some indication of the relative prevalence of the pathogenic species.

All five species of *Fusarium* studied were isolated from diseased roots collected at widely separated points in the principal soil zones of Alberta, although none of them were as prevalent as *Cylindrocarpon Ehrenbergi* (5). The three most pathogenic species, namely *F. avenaceum*, *F. arthrosporioides*, and *F. culmorum*, occurred most frequently. Also, each of these species was almost invariably the only pathogen obtained from the diseased roots from which it was isolated. *F. avenaceum* occurred with about equal frequency on alfalfa and sweet clover, and was isolated from about 20% of the diseased root samples taken from 45 different fields. This species was also isolated from the soil of several alfalfa fields and from diseased roots of *Vicia americana*. The closely related species, *F. arthrosporioides*, occurred on about 10% of the roots examined. *F. culmorum* occurred only on the roots of plants which were damaged during the summer. The most pathogenic isolates of this species were obtained from sweet clover roots in southern Alberta. *F. Poae* and *F. Scirpi* var. *acuminatum* occurred frequently on diseased roots of both alfalfa and sweet clover, but usually in association with a more virulent pathogen, such as *F. avenaceum*.

Infection Studies

INFECTION OF ROOTS BY *Fusarium* SPP. COMPARED

Representative isolates of the species which proved pathogenic in preliminary tests were studied more thoroughly in several winter and summer field experiments. Roots of Grimm alfalfa and Arctic sweet clover were inoculated and the degree of infection was estimated, as recently described (5). The results given in Tables I and II show that *F. avenaceum* was one of the most pathogenic species in both winter and summer tests. It caused a typical root rot, which was particularly severe following the winter dormancy period (Table I). Infection was first observed shortly after thawing occurred in the soil, and it was well advanced when growth started. At that time the brownish lesion formed on each inoculated root was slightly sunken toward the centre, and usually had a narrow, dark brown, or nearly black margin (Plate I, A). Lesions formed during the summer were usually less clearly defined (Plate I, C). Subsequent progress by *F. avenaceum* sometimes resulted in the rotting of the entire root system, or in the formation of large rotted areas which greatly weakened or eventually killed the plant. Sweet clover roots were generally more severely attacked than those of alfalfa. All isolates studied were pathogenic, including one obtained from a diseased root of *Vicia americana*.

F. arthrosporioides, which is closely related taxonomically to *F. avenaceum* (20), was the only other species studied which proved highly pathogenic in

TABLE I

RELATIVE PATHOGENICITY OF SPECIES OF *Fusarium* ON ROOTS OF ALFALFA AND SWEET CLOVER
IN THE EARLY SPRING
(Winter tests, 1935-36 and 1936-37)

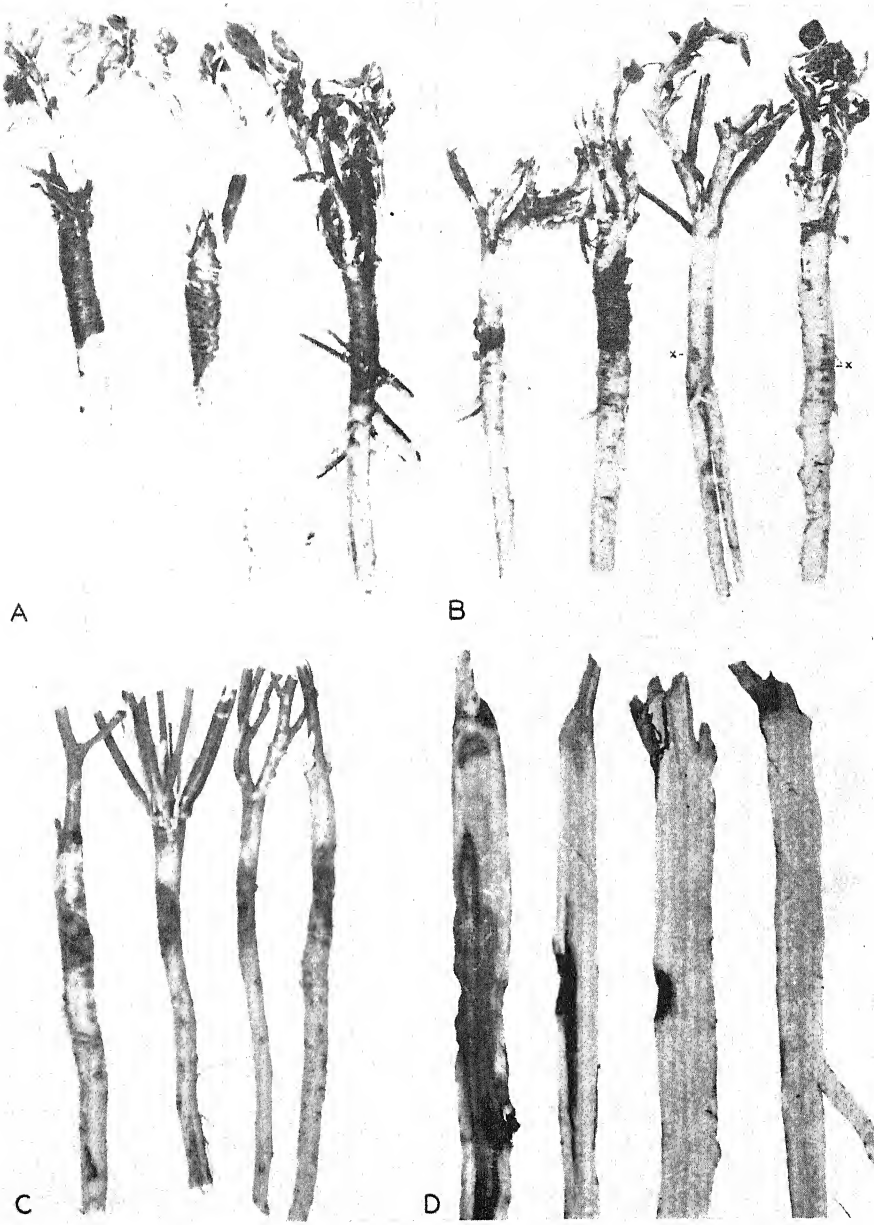
Species	Isolate		Infection rating*, %					
			Alfalfa			Sweet clover		
	No.	Source	1935-36	1936-37	Ave.	1935-36	1936-37	Ave.
<i>F. avenaceum</i>	1	Sw. clover	28	32	30	50	84	67
	6	Alfalfa	38	44	41	55	84	69
	9	Sw. clover	42	25	33	47	60	53
	14	Alfalfa	37	31	34	50	59	54
	39	Vetch		45			87	
<i>F. arthrosporioides</i>	7	Alfalfa	22	40	31	32	77	54
	35	Alfalfa		58			81	
	38	Sw. clover		50			81	
<i>F. culmorum</i>	2	Sw. clover	7	1	4	0	2	1
	37	Alfalfa		5			3	
<i>F. Poae</i>	3	Alfalfa	13	10	11	23	13	18
	4	Alfalfa	4	6	5	15	5	10
	13	Sw. clover		17			4	
<i>F. Scirpi</i> var. <i>acuminatum</i>	8	Alfalfa	5	12	8	2	15	8
	17	Alfalfa		8			5	
	34	Sw. clover		2			10	
Check plants			6	1	3	0	0	0

* Average numerical rating of 15 plants in each test.

the early spring, as well as during the growing season (Tables I and II). It produced symptoms on the roots which were indistinguishable from those caused by *F. avenaceum* (Plate I, B).

F. culmorum did not attack roots of alfalfa and sweet clover in the early spring (Table I), but during summer it was the most pathogenic species studied (Table II). Invasion of the roots by this species was particularly rapid in July and August, when sweet clover plants were often dead within two weeks after the roots were inoculated. Wilt symptoms, resembling those described by Weimer (17) for *F. oxysporum* var. *Medicaginis*, were often produced by the sudden dying of plants during summer, but examination of the roots usually revealed a typical root rot. The root lesions resembled those produced by *F. avenaceum*, but they were usually darker in color and more extensive (Plate I, C).

F. Poae and *F. Scirpi* var. *acuminatum* proved weakly pathogenic on roots of alfalfa and sweet clover in both winter and summer tests (Tables I and II). Some isolates appeared slightly more pathogenic than others and caused light to moderate infection during the summer. These species produced small, brown, and usually superficial lesions on inoculated roots (Plate I, B).



Roots of alfalfa and sweet clover artificially inoculated with *Fusarium* spp. Fig. A. Early spring infection by *F. avenaceum*. Left, two plants of Arctic sweet clover; right, Grimm alfalfa. Fig. B. Early spring infection by *F. arthrosporioides*, alfalfa and sweet clover on left, and by *F. Poae*, alfalfa and sweet clover on right. X indicates invaded areas in the latter case. Fig. C. Summer infection by *F. avenaceum*, sweet clover and alfalfa on left, and by *F. culmorum*, alfalfa and sweet clover on right. Fig. D. Influence of soil temperature and soil moisture on infection of sweet clover roots by *F. avenaceum*. Left to right: 24° C. and 60% M.H.C.; 24° C. and 30% M.H.C.; 24° C. and 15% M.H.C.; 24° C. and 5% M.H.C.

TABLE II
RELATIVE PATHOGENICITY OF SPECIES OF *Fusarium* ON ROOTS OF GROWING PLANTS OF ALFALFA
AND SWEET CLOVER
(Summer tests, 1935 and 1936)

Species	Isolate		Infection rating*, %							
			Alfalfa				Sweet clover			
	No.	Source	Test			Ave.	Test			Ave.
			1	2	3		1	3	3	
<i>F. avenaceum</i>	1	Sw. clover	26	35	21	27	54	51	47	51
	6	Alfalfa	24	17	17	19	34	29	29	31
	9	Sw. clover	22	31	17	23	26	34	34	31
	14	Alfalfa	22	25	17	21	32	23	27	27
	39	Vetch			25				35	
<i>F. arthrosporioides</i>	7	Alfalfa	17	13	11	14	36	44	42	41
	38	Sw. clover			26				52	
<i>F. culmorum</i>	2	Sw. clover	42	42	43	42	60	70	71	67
	37	Alfalfa			39				50	
<i>F. Poae</i>	3	Alfalfa	18	15	14	16	20	26	23	23
	4	Alfalfa	16	11	8	12	18	13	14	15
	13	Sw. clover		18	13			31	22	
<i>F. Scirpi</i> var. <i>acuminatum</i>	8	Alfalfa	22	21	15	19	29	32	36	32
	17	Alfalfa		18	16			29	30	
	34	Sw. clover			11				23	
Check plants			8	5	4	6	10	8	5	9

* Average numerical rating of 15 plants in each test.

PATHOGENICITY OF VARIANT TYPES

The original isolates of *F. avenaceum* from diseased roots were of the typical mycelial type, since they produced long, abundant, fluffy, white to pink mycelium on potato-dextrose agar. Subsequently, three variant types developed so frequently from this long mycelial form that they seemed to warrant detailed study. One type, occurring commonly as sectors or patches in plate culture, produced short, felted, reddish mycelium. This short mycelial type often produced sectors and patches of the original type when cultured. Cultures of the pionnotal type described by Brown (3) occurred occasionally as sectors in plate cultures of the original isolate, and were readily obtained by transferring single spores from old cultures. All attempts to make this heavily sporulating type revert to the mycelial form have so far failed. A semi-pionnotal type, with scant, matted, white mycelium intermingled with orange masses of spores, was also sometimes obtained by transfer of single spores. These variant types are of great interest from a genetical and cytological standpoint. They are being studied further, but only their relative pathogenicity will be reported at this time.

In infection experiments with alfalfa and sweet clover, the three variant types proved decidedly less pathogenic than the original isolate (Table III). Two cultures of the short mycelial type were only slightly pathogenic during

TABLE III

PATHOGENICITY OF VARIANT FORMS OF *Fusarium avenaceum* ON ROOTS OF ALFALFA AND SWEET CLOVER AS COMPARED WITH THAT OF THE ORIGINAL ISOLATE

Culture		Infection rating*, %							
		Alfalfa			Sweet clover				
Type	No.	Winter test	Summer tests			Winter test	Summer tests		
			1	2	Ave.		1	2	Ave.
Long mycelial (original)	1	32	27	23	25	84	68	44	56
Short mycelial	1b	8	11	5	8	40	21	11	16
Short mycelial	1c	8	14	11	12	47	32	13	22
Pionnotal	1d	7	19	18	18	14	49	21	35
Pionnotal	1e		14	11	12		37	14	25
Semi-pionnotal	1f	4	8	3	5	5	15	5	10
Check plants		1	0	0	0	0	12	0	6

* Average numerical rating of 15 plants in each test.

summer, but they caused moderate infection of sweet clover roots in the early spring. When re-isolations were made from the infected roots, the original long mycelial type was often obtained. The pionnotal cultures were usually somewhat more virulent than those of the short mycelial type, but the semi-pionnotal culture never caused more than a trace of infection. The pionnotal type was usually recovered from the infected root tissues. Other workers have also found that pionnotal cultures had reduced virulence. In studies of *F. avenaceum* and *F. culmorum* from carnations, Wickens (19) found that pionnotal strains were non-pathogenic, although they were derived from virulent mycelial strains.

INFLUENCE OF SOIL TEMPERATURE ON INFECTION

Under field conditions, the amount of infection produced by *F. culmorum* on roots of alfalfa and sweet clover appears to be correlated with the soil temperature. For example, two isolates were highly pathogenic in July, when the soil temperature averaged 20° C. for the period of the experiment, but they produced only light infection in a September experiment, when the soil temperature averaged 12° C. On the other hand, isolates of *F. avenaceum* and *F. arthrosporioides* caused approximately the same degree of infection in September as they did in July. These results were confirmed in a greenhouse experiment in which roots of dormant alfalfa and sweet clover plants, taken from frozen soil, were inoculated and transplanted into boxes of soil held at average temperatures of 1.5°, 12°, 17°, and 21° C. *F. avenaceum* produced moderate to heavy infection at all four temperatures, but *F. culmorum*

attacked the plants only at 17° and 21° C. This relation of temperature to infection probably explains why *F. avenaceum* can cause severe damage to the roots in the early spring, while *F. culmorum* is non-pathogenic at that time.

The influence of relatively high soil temperatures on infection of sweet clover roots by *Fusarium* spp. was studied during the summer in soil temperature control tanks. The roots of field-grown plants were inoculated and transplanted into pots containing three parts of black soil mixed with one part of sand, which were kept at temperatures of 18°, 21°, 24°, and 27° C. In the first experiment, where the soil moisture was maintained at approximately 60% of the moisture holding capacity of the soil (M.H.C.), *F. culmorum* produced nearly maximum infection at all temperatures studied (Table IV),

TABLE IV

INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE ON INFECTION OF SWEET CLOVER ROOTS BY *Fusarium avenaceum* AND *F. culmorum*

Pathogen	Expt.	Infection rating*, %							
		40% M.H.C.†				60% M.H.C.†			
		18°	21°	24°	27°‡	18°	21°	24°	27°‡
<i>F. avenaceum</i>	1					80	75	82	58
<i>F. avenaceum</i>	2	76	70	82	27	72	68	87	52
<i>F. culmorum</i>	1					97	96	94	94
Check plants	1					5	0	3	5
Check plants	2	0	0	0	1	0	0	0	1

* Average numerical rating of 30 plants.

† Soil moisture expressed as percentage of moisture holding capacity.

‡ Soil temperature in degrees Centigrade.

but *F. avenaceum* attacked the roots less severely at 27° C. than at the lower temperatures. In a second experiment, using only *F. avenaceum*, the soil moisture in duplicate sets of pots, held at each temperature, was kept at 40 and 60% M.H.C., respectively. The roots were severely rotted at 18°, 21°, and 24° C., in both dry and moist soil (Table IV). At 27° C. injury was most markedly reduced in the relatively dry soil, where only light infection occurred (Plate I, D). The indicated differential effect of soil moisture was confirmed in another experiment carried out at greenhouse temperature, where the roots were severely attacked by *F. avenaceum* in wet soil (70% M.H.C.), but were only moderately injured in soils of 55 and 35% M.H.C.

VARIETAL AND HOST RANGE TESTS

A study of varietal reaction to *F. avenaceum* has been started, since this species appears to be the most prevalent of those studied. Several commonly grown varieties of alfalfa and sweet clover, representing different species of *Medicago* and *Melilotus*, have been tested by direct inoculation of the roots. The results obtained in two winter tests and one summer test (Table V) indicate that all the species and varieties so far tested are more or less sus-

TABLE V

REACTION OF VARIETIES OF *Medicago* AND *Melilotus* AND SPECIES OF *Trifolium* TO ROOT ATTACK BY *Fusarium avenaceum*

Species and variety	Infection rating*, %			
	Winter tests			Summer tests, 1936
	1935-36	1936-37	Average	
<i>Medicago falcata</i>	12	20	16	16
<i>Medicago sativa</i> Hardistan	16	52	34	9
<i>Medicago media</i> Cossack	14	27	20	10
Grimm	12	46	29	13
Ladak	9	59	34	13
Ontario Variegated	13	62	37	10
<i>Melilotus alba</i> Arctic	27	81	54	43
Alpha No. 1	46	94	70	54
Brandon Dwarf	31	85	58	49
Grundy County	43	82	62	43
White Blossom	25	71	48	30
<i>Melilotus officinalis</i> Alborea	45	90	67	48
Yellow Blossom	28	82	55	43
Zouave	29	78	53	53
<i>Trifolium hybridum</i>	13			24
<i>T. pratense</i>	23			24
<i>T. repens</i>	23			28

* Average numerical rating of 40 plants of each variety in each test.

ceptible to attack by *F. avenaceum*. None of the species showed the consistent resistance displayed by *Medicago falcata* and *Melilotus officinalis* to attack by *Sclerotinia* sp. (13). However, all varieties of sweet clover were more severely attacked than those of alfalfa. The widely grown White Blossom and Arctic varieties had a slight but consistently lower infection rating than the other sweet clover varieties tested. Further tests are necessary before definite conclusions can be drawn.

F. avenaceum produced light to moderate infection on roots of *Trifolium hybridum*, *T. pratense*, and *T. repens* in winter and summer tests (Table V). In the summer *F. culmorum* was also pathogenic on roots of *Trifolium* spp., and usually caused more damage than *F. avenaceum*.

CROSS INOCULATION STUDIES

All five species of *Fusarium* that proved pathogenic on roots of alfalfa and sweet clover in this study also occur on cereal crops (8). In fact, *F. avenaceum* and *F. culmorum* are best known as the cause of root rot and blight

of wheat, oats, and barley (1, 15). The possible existence of host specialization in these species seemed to warrant at least a preliminary study, so cross inoculations were made on the cereals and legumes. In these experiments, isolates of *F. avenaceum* from wheat, oats, and barley, supplied by Dr. W. L. Gordon of the Dominion Rust Research Laboratory, Winnipeg, and two cultures of *F. culmorum*, isolated from wheat roots by Dr. W. C. Broadfoot of this laboratory, were tested in comparison with some of the isolates obtained from roots of alfalfa and sweet clover.

In field infection experiments, the isolates of *F. avenaceum* from oats and barley caused moderate and light infection, respectively, on roots of sweet clover, but they were only slightly pathogenic to alfalfa (Table VI). The

TABLE VI

RELATIVE PATHOGENICITY OF ISOLATES OF *Fusarium avenaceum* AND *F. culmorum* FROM LEGUMES AND CEREALS ON ROOTS OF ALFALFA AND SWEET CLOVER

Pathogen	Isolate		Infection rating*, %							
			Alfalfa				Sweet clover			
	No.	Source	Winter tests	Summer tests			Winter test	Summer tests		
				1	2	Ave.		1	2	Ave.
<i>F. avenaceum</i>	1	Sw. clover	32	27	23	25	84	68	44	56
	14	Alfalfa	31	39	25	32	59	49	32	40
	1070	Barley	4	11	5	8	33	28	8	18
	1092	Oats	10	8	8	8	68	52	27	39
	1203	Wheat	2	6	1	3	1	11	4	7
<i>F. culmorum</i>	2	Sw. clover	1	43	42	42	2	71	69	70
	B35	Wheat	3	30	41	35	3	68	51	59
	B155	Wheat	2	24	12	18	3	70	32	51
Check plants			1	8	0	4	0	12	0	6

* Average numerical rating of 15 plants in each test.

isolate from wheat was non-pathogenic, while those from alfalfa and sweet clover were moderately to highly virulent, as previously indicated. On the other hand, the isolates of *F. culmorum* from wheat were nearly as virulent as an isolate obtained from sweet clover, since they produced medium infection of alfalfa roots, and medium to heavy infection of sweet clover roots in summer tests (Table VI). In a winter test, all three isolates of *F. culmorum* were non-pathogenic.

The isolates of *F. avenaceum* and *F. culmorum* discussed above, and also one isolate of *F. arthrosporioides* from alfalfa, were tested in the greenhouse for pathogenicity on seedlings of barley, oats, and wheat. Twenty surface-disinfected seeds were planted immediately above ten grams of oat-hull inoculum in each pot of sterilized soil. Emergence notes were taken in ten days, and the experiments were concluded at the end of 30 days. At that time, the degree of infection of each root was estimated numerically, using the values

from 0 to 10, and maximum rating was given to all plants which died after emergence. The results of one experiment, summarized in Table VII, show that the isolates of *F. avenaceum* from alfalfa and sweet clover were more

TABLE VII

RELATIVE PATHOGENICITY OF ISOLATES OF *Fusarium* SPP. FROM LEGUMES AND CEREALS TO SEEDLINGS OF BARLEY, OATS, AND WHEAT

Pathogen	Isolate		Germination, %			Infection rating*, %		
	No.	Source	Barley	Oats	Wheat	Barley	Oats	Wheat
<i>F. avenaceum</i>	1	Sw. clover	74	97	72	75	31	74
	14	Alfalfa	95	95	38	49	25	88
	1070	Barley	93	93	48	41	8	65
	1092	Oats	95	100	87	35	6	31
	1203	Wheat	97	100	93	4	1	10
<i>F. culmorum</i>	2	Sw. clover	85	63	87	57	70	72
	B35	Wheat	93	76	90	57	56	62
	B155	Wheat	91	58	46	43	51	72
<i>F. arthrosporioides</i>	7	Alfalfa	97	92	80	28	31	72
Check plants			97	98	93	1	4	3

* Average numerical rating of plants in four pots.

pathogenic than those from the cereals, since they caused medium to heavy infection of barley and wheat, and light to medium infection of oats. The isolate from barley was, however, moderately pathogenic to wheat and barley, but none of the isolates from cereals caused appreciable damage to oats. Isolate 1203 from wheat was non-pathogenic on the cereals, as well as on alfalfa and sweet clover (Table VI). This culture was of the pionnotal type previously discussed, which may explain its non-virulence. The three isolates of *F. culmorum* studied were approximately equal in virulence, regardless of their origin, and produced medium to heavy infection of wheat, oats, and barley. *F. arthrosporioides* isolated from alfalfa proved highly pathogenic to wheat, and caused light to medium infection of oats and barley.

Pathological Anatomy

Since preliminary studies indicated that similar histological features were concerned in the invasion of roots by *F. culmorum* and by *F. avenaceum*, only the latter was chosen for more detailed investigation. Material showing the progressive stages of infection was obtained by inoculating non-wounded roots of alfalfa and sweet clover in the field. Sets of five roots, taken up at daily intervals after inoculation, were prepared for histological examination by the methods previously described (5).

The absence of wounds did not appear to retard penetration of the roots by *F. avenaceum*. Within two days after inoculation, hyphae were frequently observed in the basal tissues of branch roots, and in the loose outer tissues

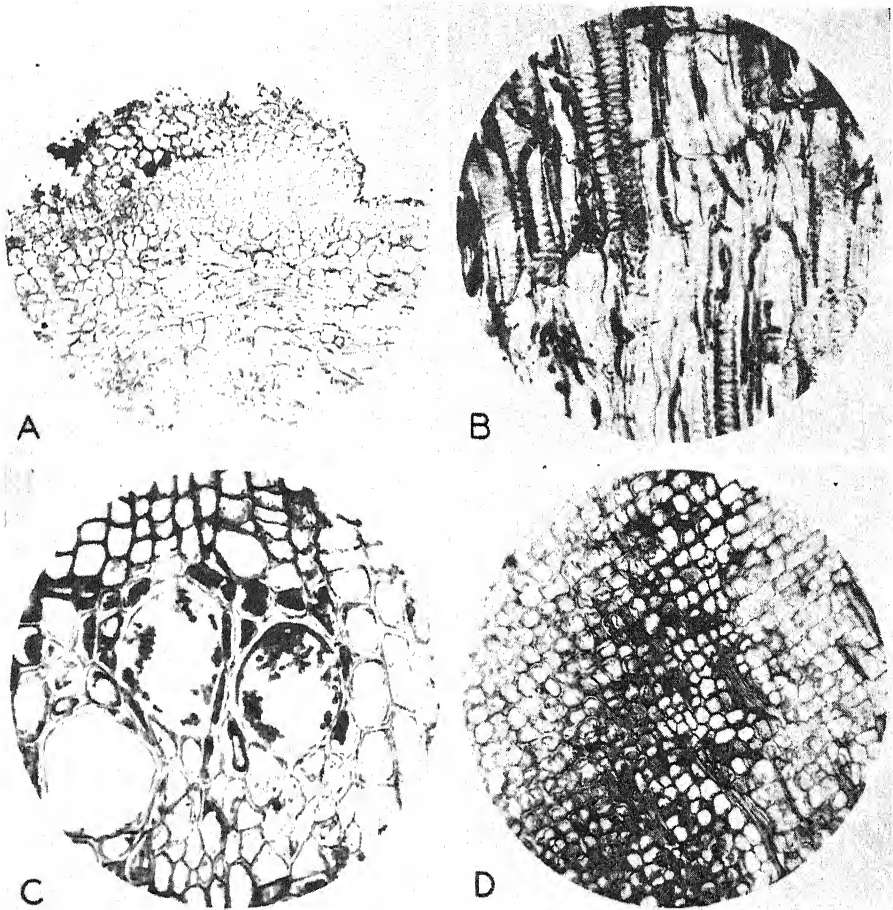


FIG. 1. Invasion of roots of *Fusarium avenaceum*. A. Hyphae starting to enter an alfalfa root through a lenticel, two days after inoculation. $\times 85$. B. Hyphae proceeding into a tap root of sweet clover by way of the connective vascular tissue of a branch root. $\times 330$. C. Cross-section of a portion of the vascular region of a tap root showing the cut ends of hyphae which are progressing longitudinally in the vessels. $\times 330$. D. Line of demarcation at border of an old lesion. Uninvaded area at right. $\times 130$.

of lenticels (Fig. 1, A). The thin cork layers below these points usually offered but little resistance to the pathogen. When infection occurred at the base of a branch root, the hyphae often passed into the tap root by way of the connective vascular tissues of the branch root (Fig. 1, B). Apparent cases of direct penetration of the outer cork layer of the root were also observed, but they were relatively rare.

The phloem parenchyma, phloem, cambium, xylem, and central portion of the tap root were all readily invaded by *F. avenaceum*. The hyphae proceeded singly through and between the cells, or they aggregated into strand-like masses which ruptured their way through the tissues. Longitudinally, they appeared to progress most rapidly in the vascular tissues

(Fig. 1, C). This probably explains the rapid rotting commonly caused in the central portion of the root (Plate I, D). *F. avenaceum* also appeared to have an advance toxic action, since the cells were often progressively disorganized for some distance ahead of the area where hyphae were visible. Similarly disorganized cells occurred in the dark border formed at the margin of lesions, when active progress of the pathogen ceased (Fig. 1, D).

Physiological Studies

INFLUENCE OF TEMPERATURE

The temperature relations of *Fusarium* spp. were studied in the hope that they might throw some light on the results obtained in the infection experiments. Representative isolates, freshly transferred to plates of potato-dextrose agar, were incubated in quadruplicate at temperatures ranging from -2° to 36° C. Their relative growth was essentially the same in several different series, so the results of only one experiment are presented (Table VIII, Fig. 2).

TABLE VIII

INFLUENCE OF TEMPERATURE ON GROWTH OF *Fusarium* SPP. ON POTATO-DEXTROSE AGAR

Species	Average diameter of colonies in mm. at different temperatures*											
	3°	5°	8.5°	10.5°	14°	17°	20°	24°	27.5°	29.5°	32°	34°
<i>F. avenaceum</i>	0	7	10	15	26	33	37	43	38	21	0	0
<i>F. arthrosporioides</i>	0	6	8	12	19	28	33	38	34	14	0	0
<i>F. culmorum</i>	0	0	0	12	22	36	62	73	73	36	9	0
<i>F. Poae</i>	0	6	8	9	14	21	26	26	18	11	0	0
<i>F. Scirpi</i> var. <i>acuminatum</i>	0	0	0	7	12	15	25	30	28	15	0	0

* Average temperatures in degrees Centigrade for the three days during which the plates were incubated.

F. avenaceum grew well at a wide range of temperatures, with an optimum at about 24° C., and a maximum at 34° C. It started growth in five days at 1° C., and even grew slowly on frozen agar at -2° C. *F. arthrosporioides* had a similar temperature relation, but it grew more slowly than *F. avenaceum* at all temperatures. *F. Poae* had the lowest optimum and maximum temperatures of any species studied, namely 20° to 24° C., and 32° C., respectively. The minimum for this species was about -2° C.

The other two species developed best at relatively high temperatures. *F. culmorum* had a minimum at 3° C., and grew very slowly at temperatures below 10° C. This species made very rapid growth between 15° and 30° C. It had an optimum at 24° to 27° C., and a maximum at 34° to 36° C. *F. Scirpi* var. *acuminatum* also failed to grow at a freezing temperature, but it did develop slowly at 3° C. Best growth of this variety occurred at 20° to 28° C., with an optimum at 24° C., and a maximum at 34° C.

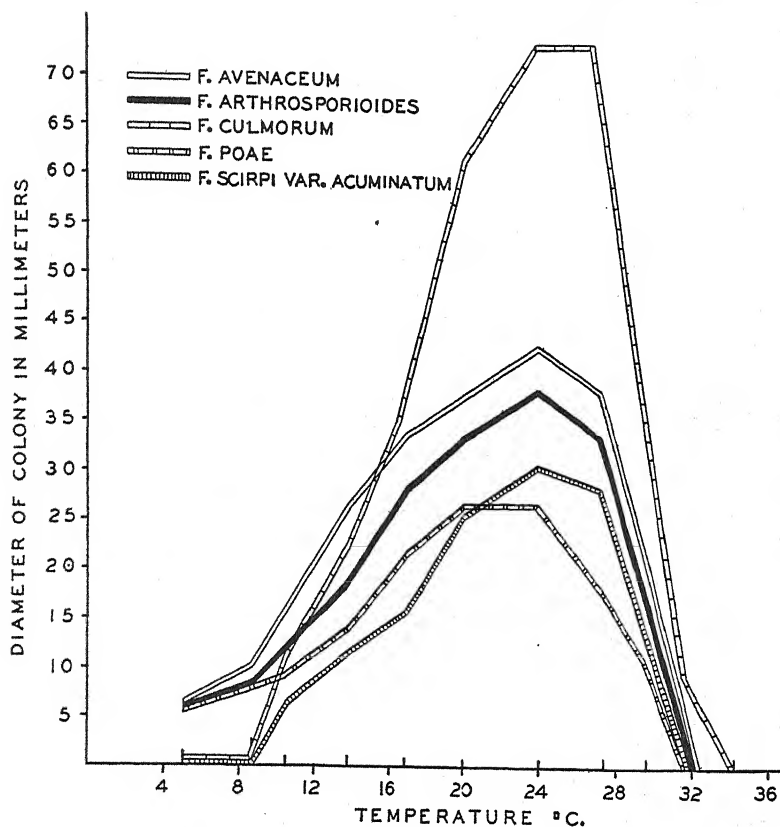


FIG. 2. Growth of *Fusarium* spp. on potato-dextrose agar, after incubation for three days at temperatures ranging from 5° to 34° C.

INFLUENCE OF HYDROGEN ION CONCENTRATION

Fusarium spp. were grown on buffered agar and liquid media adjusted to a wide range of pH values by the methods previously described (5). Their growth on Czapek's synthetic agar is typical of the results obtained (Table IX).

TABLE IX

INFLUENCE OF HYDROGEN ION CONCENTRATION ON GROWTH OF *Fusarium* SPP. ON CZAPEK'S SYNTHETIC AGAR

Species	Average diameter of colonies in mm. at different pH values									
	2.8	4.0	4.6	5.1	5.8	6.2	6.9	7.7	8.4	9.5
<i>F. avenaceum</i> *	37	70	71	70	72	50	53	60	51	47
<i>F. arthrosporioides</i> *	39	65	65	64	67	54	58	62	52	49
<i>F. culmorum</i> †	15	48	51	53	58	55	60	55	54	29
<i>F. Poae</i> *	23	53	55	57	58	56	56	56	56	47
<i>F. Scirpi</i> var. <i>acuminatum</i> *	12	45	49	50	33	44	45	46	45	26

* Incubated for six days.

† Incubated for three days.

All species studied grew at pH values ranging from 2.8 to 9.5, but they developed particularly well in the alkaline range. With *F. avenaceum* and *F. arthrosporioides*, the best growth occurred at pH 5.8 and pH 7.7 in potato-dextrose and Czapek's synthetic solutions, and on Czapek's agar (Table IX). There was distinctly less growth at pH 6.2, indicating an isoelectric point in that region. Further evidence of this was obtained in spore germination studies where 90% of the spores germinated at pH values of 5.2 and 6.8, while only 30% germinated at pH 6.0.

More variable results were obtained with the other species studied. *F. culmorum* grew best at pH values of 5.8 and 6.9 on Czapek's agar and liquid media, but had only one optimum, at pH 6.3, on the potato-dextrose media. Best growth of *F. Poae* occurred at pH 6.2 on Czapek's media, and at pH 5.2 on potato-dextrose media. In all media employed, *F. Scirpi* var. *acuminatum* grew best at pH 5.1 and at pH 6.9 to 7.7, with less growth at pH 5.8. Growth of *Fusarium* spp. generally tended to make the liquid media more alkaline.

The ability of these species of *Fusarium* to grow at such a wide range of hydrogen ion concentrations indicates that they should be able to thrive in most cultivated soils. They will probably develop best in soils with the neutral or slightly alkaline reaction suited to growth of alfalfa and sweet clover.

INFLUENCE OF CARBON DIOXIDE

There is a possibility that poor soil aeration and consequent accumulation of carbon dioxide around the roots in the early spring may be factors in increasing the susceptibility of alfalfa and sweet clover at that time to attack by *F. avenaceum* and other root-rotting fungi. Lundegårdh (9) found that wheat seedlings were more severely attacked by *Fusarium* spp. when exposed to various concentrations of carbon dioxide than in ordinary air. He suggested that a slowly melting snow covering or a high water content might increase the carbon dioxide content of the soil. During the present investigation an opportunity was not afforded for a study of these soil relations, but experiments were started on the influence of carbon dioxide upon the growth of *Fusarium* spp. in pure culture.

The containers used in these experiments consisted of two coffee tins, of one pound capacity, soldered together and made airtight. Two outlets were provided in the form of short tubes soldered near the top and bottom of each container. A toy balloon of good capacity was sealed on the lower tube to allow for expansion of the gases. Freshly transferred plate cultures, in triplicate, were used. The lid of each plate was lifted slightly by means of an iron wire placed inside. When ready, the plates were stacked in a wire frame and lowered into the container, which was then completely sealed. Carbon dioxide from a commercial tank, measured by water displacement in a burette attached to a levelling bulb, was forced into the container through the upper connection. The previously calculated air content of the container and connections determined the amount of carbon dioxide which it was neces-

sary to add to produce the required concentration. Atmospheric pressure was maintained through expansion of the balloon and, when necessary, a second balloon was sealed on the top connection. Proper mixture of gases was maintained by a gentle pressure applied alternately to the two balloons at regular intervals. Control cultures were placed in similarly sealed cans containing ordinary air. Atmospheres with a carbon dioxide content ranging from normal to 20% were employed. Most of the experiments were conducted at room temperature, but one set of containers was incubated at 8° C. to determine the possible influence of temperature. The data from one experiment (Table X) are typical of those obtained.

TABLE X
INFLUENCE OF CARBON DIOXIDE CONCENTRATION ON GROWTH OF *Fusarium* SPP. ON POTATO-DEXTROSE AGAR

Species	Average diameter of colonies in mm.							
	Room temperature						8° C.	
	Air	CO ₂ , %					Air	CO ₂ , %
		2.5	5	10	15	20		15
<i>F. avenaceum</i> †	52	49	49	51	51	49	33	29
<i>F. arthrosporioides</i> †	40	38	36	38	36	39	30	22
<i>F. culmorum</i> *	90	90	86	85	83	79	46	27
<i>F. Poae</i> †	28	29	27	28	27	23	17	14
<i>F. Scirpi</i> var. <i>acuminatum</i> †	42	42	39	40	34	30	22	13

* Incubated for three days.

† Incubated for seven days.

At room temperature, carbon dioxide concentrations up to 20% had little or no effect on the growth of *F. avenaceum* and *F. arthrosporioides*. These species were, however, slightly retarded by 15% carbon dioxide at 8° C. Growth of *F. Poae* was also very slightly retarded by the higher concentrations of carbon dioxide. On the other hand, *F. culmorum* made consistently less growth as the carbon dioxide concentration of the atmosphere was increased. At 8° C., 15% carbon dioxide markedly retarded the growth of this species. Atmospheres containing 15 and 20% carbon dioxide also had a distinct retarding influence on *F. Scirpi* var. *acuminatum*.

These results are in general agreement with those reported by other workers. Brown (2) noted that carbon dioxide had a greater retarding effect on spore germination and growth at a low temperature than at a high temperature. He suggested that this was partly due to the increased solubility of carbon dioxide in water at low temperatures. Fellows (6) found that growth of *Ophiobolus graminis* was slightly retarded by 18% carbon dioxide, the highest concentration employed. With *Phymatotrichum omnivorum*, Neal and Wester (10) reported that growth was retarded only by concentrations of carbon dioxide greater than 25%. Lundegårdh (9) did not observe any

retarding influence of carbon dioxide on the growth of *F. avenaceum*, *F. culmorum*, and other species of *Fusarium*, but 7% was the highest concentration which he employed. Hence, it appears as if relatively low concentrations of carbon dioxide have little, if any, effect on the growth of *Fusarium* spp. It seems doubtful whether concentrations as high as those which retarded growth in the present study will occur under natural conditions in the soil.

Discussion

Although the species of *Fusarium* studied herein are apparently not as prevalent as certain other pathogens, they do contribute to the root-rot damage suffered by alfalfa and sweet clover in Alberta. The closely related species *F. avenaceum* and *F. arthrosporioides* appear most important, since they occur commonly, and can cause severe damage both in the early spring and during the summer. *F. culmorum* is very virulent in the summer, but it is apparently incapable of attacking the roots in the early spring. The relatively light infection caused at all times by *F. Poae* and *F. Scirpi* var. *acuminatum* indicates that they are weak parasites or saprophytes on roots of alfalfa and sweet clover. This is further suggested by the frequent association of these species with a more virulent pathogen. A final evaluation of the relative importance of *Fusarium* spp. as root parasites of alfalfa and sweet clover in Alberta must await further study.

The relative virulence of *F. avenaceum*, *F. arthrosporioides*, and *F. culmorum* under different conditions appears to be closely correlated with the temperature relations of these species. Since *F. culmorum* was unable to grow at temperatures near freezing, it did not attack roots of alfalfa and sweet clover in the early spring. On the other hand, *F. avenaceum* and *F. arthrosporioides* grew well at a wide range of temperatures, and caused heavy infection in the early spring, as well as during the growing season. These species developed best at about 24° C., while *F. culmorum* had an optimum at 24° to 27° C. This probably explains why *F. culmorum* usually caused slightly higher infection than the other species during the summer, but became less pathogenic with lowering of the soil temperature towards fall. Further evidence of this relation of temperature to infection was obtained in the greenhouse studies, where *F. avenaceum* caused more infection at 24° C. and lower temperatures than at 27° C., while *F. culmorum* did not attack the plants at low temperatures, but was highly virulent at 18° to 27° C. These results are in general agreement with those reported by other workers. Simmonds (15) found that *F. culmorum* from oats had an optimum of 24° to 28° C. for growth in pure culture, and produced a greater degree of infection at 18° to 30° C. than at 8° to 15° C. Bennett (1) concluded that *F. culmorum* was better suited to high temperatures than *F. avenaceum*.

A singular lack of specificity was shown by the isolates of *F. avenaceum*, *F. arthrosporioides*, and *F. culmorum* obtained from alfalfa and sweet clover roots, since they also attacked roots of *Trifolium* spp. and seedlings of wheat, oats, and barley. Furthermore, certain isolates from the cereal crops were

pathogenic on roots of alfalfa and sweet clover. Further study of these and other isolates is necessary, since Tu (16) found two physiologic races of *F. avenaceum*, and three of *F. culmorum* among the forms causing head blight of cereals. However, the present results indicate that the use of legumes or cereals in crop rotation may have certain limitations in reducing the root-rot damage of either crop caused by *Fusarium* spp. Moreover, the fact that isolates of *F. avenaceum* from *Vicia americana* were pathogenic on roots of alfalfa and sweet clover suggests that wild hosts in virgin soil may harbor and increase pathogenic species of *Fusarium*, which may subsequently menace legumes or other cultivated crops.

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HYBRIDIZATION OF *TRITICUM* AND *AGROPYRON*

III. CROSSING TECHNIQUE¹

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Abstract

Crossing technique is discussed with respect to coincidental flowering of parental plants, emasculation, bagging, tagging, marking, collection of pollen and pollination.

Data are presented which demonstrate that stigmas of emasculated wheat florets retain their receptivity, and may be successfully pollinated, a week or more after the normal time of flowering. It is also shown that wheat and *Agropyron* pollen may be stored under room conditions for a day or two without appreciable reduction in viability. These points are discussed in relation to crossing technique.

Introduction

During the past three years the authors have been engaged in research that involves the crossing of *Triticum* and *Agropyron*. Since the first year's results were reported (1) the work has been extended to include nine *Triticum* species and sixteen *Agropyron* species, and has progressed to a point where about seventy thousand florets have been emasculated and pollinated. In nearly all crosses *Triticum* has been the maternal and *Agropyron* the paternal parent. Crossing technique, based on this experience, is discussed here with two ends in view: first, to give workers directly interested in the hybridization of *Triticum* with *Agropyron* a more detailed account of the technique than was previously reported; and, second, to give general information, suggestions, and hints that may be generally applicable to hybridization in the *Gramineae* and perhaps in other families. It is assumed that the reader is an experienced hybridizer. Readers who desire more complete accounts of crossing technique than that given here are referred to the work of Hayes and Garber (3, pp. 120-129) for crops in general, to that of Florell (2) for cereals, and to that of Jenkin (4) for herbage grasses.

Obtaining Coincidental Flowering of Parental Material

It is sometimes difficult to make the flowering periods of parental species coincide. In crosses involving two annual forms a number of successive sowings of one of the parents is all that is necessary, and when the hybridizer is familiar with both parents the necessary adjustment may be made by single sowings. Where winter annuals, biennials or perennials are involved, however, more difficulty may be experienced.

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The following conclusions and recommendations are based on experiences in the manipulation of the time of flowering of winter wheats and perennial *Agropyrons*:

(1) Practices and treatments, such as different times of sowing, cutting-back, etc., carried out during the previous year do not appreciably change the time of flowering.

(2) Cutting-back of wheats and grasses in the spring has practically no effect on the time of flowering, but may be very detrimental to subsequent growth.

(3) Midwinter sowing of winter wheat in the greenhouse, followed by exposure of the resulting plants to moderate cold in the late winter or early spring, and transplanting to the field as early as possible, has been reasonably successful. This practice, however, has the disadvantage of being laborious, requiring greenhouse space, and of producing, in our experience, a somewhat poor stand in the field, due probably to the wet, unfavorable, soil conditions generally existing at the time of early spring transplanting.

(4) The use of vernalized seed is believed to afford the most satisfactory as well as the simplest means of controlling the time of flowering in winter-annual, biennial and perennial plants. For early plants (of winter wheat in our case) the seed should be vernalized in late winter and started in the greenhouse. For later plants the seed should be vernalized just prior to sowing, which should be done at about the time of the earliest spring wheat sowings. It is often characteristic that a stand from vernalized seed will head over a considerable period of time, an advantageous condition for crossing work. In our experience winter wheats grown from vernalized seed sown in the first week of May have provided a continuous supply of heads for emasculation throughout July.

(5) It should be borne in mind that any of these practices may be more or less detrimental and that, since the paternal parent should be favored culturally in order that there may be an abundance of pollen, it is preferable that flowering-time manipulations should be practised upon the maternal parent.

Emasculation

Efficient emasculation involves the complete removal of unbroken anthers from the floret in such a way as to minimize injury, direct or indirect, to the female reproductive organs, and at a time known to be approximately a given number of days in advance of normal flowering. The accomplishment of these requirements is almost entirely a matter of developing skill in the actual operation and in the observation of the proper stages in the plant. There are, however, a number of details about which something useful may be said.

It is usually necessary to adapt standard forceps to emasculation work by grinding or filing the points and by altering the tension. We prefer a straight type with corrugated handles and a point approximately 2 mm. wide. The points are ground down in long, tapering fashion until they are 1.0 to 1.5 mm.

in width and, when examined in sideview, show a nearly straight taper to a fairly sharp point. The tension is usually too strong in standard forceps. This may be weakened by inserting a tapering penholder near the fusion of the handles, forcing them apart, and then bending the handles slightly by pressing the points toward each other with the fingers. A very weak tension is perhaps even more tiring than one too strong. It may be corrected by spreading the handles. The correct tension is one that permits a firm, steady grip without closing the points, yet permits closing the points with a slight pressure.

The standard, straight-bladed, sharp-pointed, dissecting scissors are perfectly adapted to the removal of spikelets, clipping of awns, etc.

Each operator should have a comfortable stool about a foot high, containing a drawer or compartment for instruments, supplies, etc. A folding camp stool to which a suitable pocket had been attached has proved to be very comfortable and convenient.

We usually pollinate wheat florets three days after emasculation, and for this interval select heads the most mature florets of which appear to be two days removed from flowering. The extra day permits most of the less mature florets to become receptive before pollination. The time to emasculate is determined by two factors: the anther must not have matured to the point where there is danger of dehiscence in removal, and the time of flowering must not be too far away to permit a reasonably accurate estimation of the date.

The number of spikelets and florets to be removed from the spike will depend upon circumstances. Ordinarily maximum efficiency is attained if the extreme tip spikelets and a somewhat larger number of extreme basal spikelets are removed, together with the central florets of the remaining spikelets. The range of flowering time in the florets retained should cover only a day or two; ordinarily the optimum practical condition is to have the more mature florets become receptive a day prior to pollination, and the remainder of the florets becoming receptive at the time of or just prior to pollination. If, because of a dearth of material, it is important to cross a maximum number of florets from each head, practically every spikelet (comprising the lateral and probably the larger central florets in most of the spikelets) may be used. Since the range of flowering time in such spikes may cover several days it is advisable to pollinate two or three times, or at least to delay pollination until most of the later florets have become receptive. (While the preceding discussion is based upon the spike-type inflorescence, the points brought out are also more or less applicable to the panicle-type inflorescence.)

Bagging, Tagging, Marking

The glassine envelope is probably the best form of bagging. Glassine paper, while pollen-tight, permits sufficient passage of air and moisture to provide a favorable environment for the enclosed head. Cellophane envelopes, on the other hand, are highly unsatisfactory as they do not permit sufficient

aeration and evaporation. A common mistake in the use of the glassine envelope is to have it far too large. We have been using an envelope size $6 \times 1\frac{1}{2}$ in. on wheat and have found it perfectly satisfactory. (A common size for wheat seems to be $7 \times 3\frac{1}{2}$ in.) The use of large envelopes causes the loss of a great many heads through breakage from wind and rain.

String tags are used to label the heads and are placed well down on the stem to prevent beating about in the wind. It is important that writing should be done with a sharp, medium hard (H or 2H) pencil, using sufficient pressure to make very distinct marks.

Where heads are emasculated as they reach the right stage on successive days and where this is continued over a period of several days, a great deal of time may be saved during pollination by a mark that will indicate at a glance the heads emasculated on a given day. For this purpose we use colored strings, 6 to 8 in. long, for fastening the glassine envelopes to the emasculated heads, a different color being used each day. Thus, when pollinating, we can find immediately the heads emasculated three days previously, even though they are distributed among several hundred other bagged heads. When the envelopes are replaced after pollination the colored strings are replaced by white ones. Since we usually pollinate three or four days after emasculation, only four colors are necessary (though other colors are held in reserve in case pollination is delayed without a corresponding delay in emasculation). Various biological stains are used to dye the strings, among the more successful being: orange G (1% aqueous solution), methyl blue (1% aqueous solution), and acid fuchsin (1% dissolved in 70% ethyl alcohol).

Duration of Stigmatic Receptivity

It is apparently a common belief among hybridizers that the stigma retains its receptivity for only a very short period, perhaps for a few hours or a day or two, depending on conditions. As far as is known no data have previously been published on the period of time over which stigmas retain receptivity, although Florell (2) mentions obtaining good seed sets six to eight days after emasculation in wheat crosses.

The data presented in Table I serve to indicate the duration of stigmatic receptivity in emasculated winter and spring wheats as determined by the relative seed sets upon pollination with *Agropyron glaucum*. Emasculation was performed two or three days before normal flowering time. All spikes were cut down to 20 florets (the two lateral florets of five central spikelets on either side of the rachis), which were in all probability within one day of being at the same stage with respect to flowering time. Hence, it is believed that all stigmas should have been receptive within three or four days after emasculation. Therefore, the data in Table I demonstrate that, under the conditions of the experiment, unpollinated stigmas of emasculated wheat florets remained highly receptive and functional for at least six or seven days after the time of normal flowering.

TABLE I
COMPARISON OF NUMBERS OF HYBRID SEEDS SET IN A SERIES OF WHEAT VARIETIES POLLINATED WITH *Agropyron glaucum* AFTER ELAPSED PERIODS
OF FROM TWO TO ELEVEN DAYS AFTER EMASCULATION

Maternal parent (wheat)	2-3 days			4-5 days			6-7 days			8-9 days			10-11 days		
	Florets	Seeds	%	Florets	Seeds	%	Florets	Seeds	%	Florets	Seeds	%	Florets	Seeds	%
Kharkov	—	—	—	60	4	6.66	460	163	35.43	40	28	70.00	40	24	60.00
White Odessa	140	43	30.71	220	10	4.55	660	120	18.18	140	0	0.00	—	—	—
Dawson's G.C.	—	—	—	140	52	37.14	460	88	19.13	60	0	0.00	40	14	35.00
C.D. 1435	—	—	—	300	33	11.00	400	55	13.75	80	3	3.75	—	—	—
Minhardi	—	—	—	100	8	8.00	500	91	18.20	—	—	—	—	—	—
Secalotricum	—	—	—	140	6	4.29	460	86	18.70	—	—	—	—	—	—
Minturki	—	—	—	100	5	5.00	640	8	1.25	60	3	5.00	—	—	—
Lutescens 0.329	100	2	2.00	140	2	1.43	480	3	0.63	—	—	—	—	—	—
Lutescens 0.62	180	2	1.11	560	6	1.07	840	44	5.24	—	—	—	—	—	—
Mindum	120	20	16.67	460	23	5.00	660	30	4.55	—	—	—	—	—	—
C.A.N. 1835	200	2	1.00	460	5	1.09	660	4	0.61	—	—	—	40	0	0.00
Vernal emmer	80	0	0.00	80	14	17.50	60	0	0.00	—	—	—	—	—	—
Combined data	820	69	8.41	2,760	168	6.09	6,280	692	11.02	380	34	8.95	120	38	31.67

During the time of the experiment the temperature was warm to moderately hot, while the humidity was at all times moderately high. It is believed probable that the period of stigmatic receptivity would be considerably shorter under extremely hot, dry conditions such as are experienced on the prairies.

With one exception, no pollinations were made later than 11 days after emasculation. The exception was one 20-floret spike of Minturki pollinated 15 days after emasculation. No seeds were set. Florell (2) states that in rare cases hybrid seed was obtained from pollinations made 23 to 25 days after emasculation.

The fact that, under favorable conditions, stigmas may remain highly receptive for a week or more has direct bearing on crossing technique. It is the common practice to pollinate when the first stigmas in the head become receptive; but, since the florets of an emasculated head practically always have a range of a day or two in the time at which they become receptive, and since it is doubtful that dehiscent pollen may remain viable for more than a few hours in the floret, it would appear advisable to delay pollination until all florets have become receptive. This is in accord with our practice of emasculating two days (estimated) before flowering, but pollinating three days after emasculation.

Another instance when delayed pollination would be advisable is in crosses involving similar varieties in which selfed seed cannot be distinguished from crossed seed and, more particularly, where the respective characters are not sufficiently differential to distinguish selfed from hybrid plants in the F_1 . In such crosses one could eliminate selfed seeds arising from contamination at the time of emasculation by delaying pollination until the seeds had begun to form, indicating that self fertilization had occurred.

Collecting Pollen, Pollination

In our hybridization work with perennial grasses and cereals the pollen is commonly collected in a more or less dehiscent condition using 600 or 800 cc. beakers. The use of beakers is to be recommended for a number of reasons; they are handy for collecting, pollinating and storing; they permit adequate aeration of collected pollen, thus preventing "clumping" of pollen grains; and they enable one to pollinate a maximum number of heads with a given amount of pollen. Aeration of pollen, particularly when many anthers are present, is very important under conditions at Ottawa. Pollen grains and anthers transpire sufficiently to increase the humidity to the point where, unless there is adequate aeration, the grains will stick together in clumps of perhaps several hundred. Clumped pollen gives very poor results. Even in open beakers we try to keep the pollen well dispersed by letting it adhere as much as possible to the sides of the beaker when collecting, for, except on days of particularly low humidity, the concentration of pollen in the bottom to any depth will cause clumping. Glumes and anthers falling into the beaker are removed at once, since they give off moisture which may cause clumping. Rainy or excessively humid days are very unfavorable for pollination work.

When collecting pollen from *Agropyron* species artificial stimulation of flowering by "stroking" was commonly practised. (The stroking method consists in stimulating heads which are nearly ready to flower by a few upward strokes with the fingers. Within a few minutes after such treatment the glumes will spread apart and the anthers extrude.) These species characteristically flower very abruptly and over a very short period each day. Since one or two workers were obliged to collect from several species, often in different fields, it was necessary to stimulate flowering artificially some time before flowering would have naturally occurred in order that the rounds could be made successfully. This practice was also useful when collecting from a single species as it made waiting for pollen unnecessary and prevented the possibility of not being on hand when flowering occurred. Pollen is collected by inserting the heads with extruded anthers into the mouth of the beaker where they are rolled or shaken by the fingers to burst or dislodge the anthers.

It is not advisable to induce flowering artificially at a time or under conditions too far removed from the normal. It is often possible to cause anther extrusion several hours before normal flowering time, or on wet, cool days; but pollen thus obtained is sticky, has a tendency to clump, and is of doubtful value in pollination.

Pollination is a more critical operation than emasculation, in that the pollen itself is subject to many conditions which may be, and often are, adverse. The success attending pollination on different days may vary from a negative result to a nearly perfect seed set, even though the same methods and plant materials are used. It is known that such factors as very high humidity, premature stroking, insufficient aeration of pollen, etc., contribute greatly to decreases in crossing success; but it is far from possible fully to account for the differences observed. In short, one cannot be to any degree certain that a given pollination represents a maximum opportunity for fertilization. Perhaps a pollination made on another day, under apparently identical conditions, with a different collection of pollen from the same species, would provide a much better opportunity. Since pollination is ordinarily much easier than emasculation, it may be better, therefore, for an operator to emasculate fewer heads and take time to pollinate all heads two or three times, on successive days or at two-day intervals. We have practised repeated pollinations to a considerable extent and have observed improved seed sets as a result.

Repeated pollinations are also useful in providing fresh pollen for late maturing stigmas which may not have been receptive at the time of the first pollination. Jenkin (4) practised repeated pollinations on certain grasses, especially for this reason. In our own material, however, we feel that the value of repeated pollinations is related in the main to pollen rather than to stigmatic factors.

For applying pollen to the stigmas, sable hair brushes, such as are used for water color painting, have been found to be perfectly satisfactory. We use Winsor and Newton's (England) Series 16, Numbers 1 or 2, which have pointed brushes about 11 mm. long and 1.5 mm. in diameter at the base.

The pollen dust is merely brushed on to the stigmas in sufficient amounts to be noticeable on the brush, one or two florets being pollinated with each brushful. Where there is an abundance of pollen it is probably advisable to imitate nature in being very lavish; but where pollen is scarce it should be used sparingly in order that a maximum number of heads may be pollinated. If the stigmas are not exposed, the glumes may be spread apart by the tip of the brush handle (which should be cut wedge-shaped for this purpose) and held apart with the fingers while pollen is applied to the exposed stigmas.

Methyl alcohol is used for sterilizing beakers and brushes. This chemical is completely effective and, since it evaporates very quickly, there is practically no delay as a result of sterilization.

Storage of Pollen

From time to time in our hybridization work there have arisen situations in which a temporary lack of pollen has emphasized the need for information on the possibility of some simple way of storing pollen for a few days. A difficulty of this kind frequently arose because of afternoon showers which more or less prevented pollination while permitting emasculation, done in the forenoon, to proceed unhampered, resulting in a disruption of our schedule and a superabundance of emasculated heads. We were interested in storing surplus pollen collected on bright days to be used for morning pollination when the number of emasculated heads became excessive. This, ordinarily, would entail storage for only one to three days. Since we could find no literature on the point, an experiment was carried out to test the possibility of such storage.

Pollen of *Agropyron elongatum* and of *Triticum turgidum* was collected on three successive days and stored in beakers under room conditions in a ventilated cabinet. On the fourth day all three stored collections of each kind of pollen, together with fresh check collections, were applied to florets which had been emasculated three days previously. The seed-set results of this experiment are summarized in Table II.

From the results obtained it would appear safe to conclude that pollen of wheat and of *Agropyron* may be stored under room conditions for a day or two without undergoing any great reduction in viability. There is, however, a marked decrease in the number of seeds set when pollen stored three days was used, which indicates that a decrease in viability may begin at about the third day of storage.

In discussing the viability of stored pollen there arises the question of how long dehiscent pollen may be expected to remain viable on immature stigmas in the floret. It seems doubtful that pollen would remain viable in an ungerminated condition for any appreciable length of time on the stigma owing to moisture and other germinative factors existing in the floret. Our own observations and those of Florell (2) demonstrate the fact that in certain crosses seed may be produced by pollinating immature stigmas; but it is believed that this is due to the more or less immediate germination of pollen

TALE II

RELATIVE VIABILITY OF FRESH POLLEN AND OF POLLEN STORED FROM ONE TO THREE DAYS
AS INDICATED BY SEEDS OBTAINED WHEN USED IN CROSS POLLINATION

Cross	Pollen	Fresh			Stored 1 day		
		Florets	Seeds	%	Florets	Seeds	%
Kharkov \times A. elongatum	A. elongatum	142	2	1.41	170	3	1.76
Yaroslav \times A. elongatum	A. elongatum	142	3	2.11	168	1	0.60
T. turgidum \times T. vulgare	T. turgidum	48	6	12.50	86	4	4.65
Combined data		332	11	3.31	424	8	1.89

Cross	Pollen	Stored 2 days			Stored 3 days		
		Florets	Seeds	%	Florets	Seeds	%
Kharkov \times A. elongatum	A. elongatum	146	7	4.79	154	1	0.65
Yaroslav \times A. elongatum	A. elongatum	146	3	2.05	156	2	1.28
T. turgidum \times T. vulgare	T. turgidum	88	4	4.55	88	0	0.00
Combined data		380	14	3.68	398	3	0.75

and penetration of the pollen tube, rather than to a delay in germination until the stigmas mature. The pollination of immature stigmas however has not, in our experience, been sufficiently successful to be recommended, particularly in wide crosses.

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CHEMICAL WEED KILLERS

V. RELATIVE TOXICITY OF SELECTED CHEMICALS TO PLANTS GROWN IN CULTURE SOLUTION, AND THE USE OF RELATIVE GROWTH RATE AS A CRITERION OF TOXICITY¹

By W. H. Cook²

Abstract

Substances previously found to be highly toxic when applied to annuals as a spray were also found to be most toxic when added to culture solution. The results by the two methods, however, do not agree as far as the less poisonous chemicals are concerned, certain substances being comparatively more toxic in culture solution than as a spray, and *vice versa*. These discrepancies can be explained by the fact that the dosage in culture solution was varied by adjusting the concentration, whereas in the spraying test it was varied by altering the volume of spray.

The time between treatment and death of the plant generally decreases as the dosage is increased over a limited dosage range, but varies with different chemicals, and appears to be independent of their inherent toxicity.

The size of the plant is seriously reduced at dosages that produce no mortality. The final weight, however, was unsatisfactory as a criterion of toxicity since it was extremely variable. The interfering factors affecting the final weight were taken into account by computing the relative growth rate. The curve relating growth rate and dosage is slightly concave upwards when the dosage is plotted on an arithmetical scale and linear when plotted on a logarithmic scale. The position and slope of the line depends on the chemical. The standard error of duplicate tests increases as the growth rate decreases. On the average, complete mortality occurred at a growth rate of -2.44% per day under the conditions of these experiments, but this is subject to variation due to differences between duplicates, chemicals, and series (plants grown at different times).

Analyses of the culture solutions containing chlorates showed that the amount of chlorate taken up by the plant increased with the concentration present in the culture solution. Nevertheless, only a small, relatively constant proportion of the chlorate present was taken up by the plant at all concentrations.

Introduction

A knowledge of the true toxicity of substances used as weed killers should lead to a better understanding of the factors limiting their efficacy. When a toxic solution is applied as a spray to plants grown on soil, the efficacy of the treatment depends, not only on the inherent toxicity of the chemical, but also on the quantity retained by the leaves, and the detoxicating effect of the soil. It has been shown in previous publications (5, 6, 7) that the quantity of chemical retained by the leaves is dependent on the volume and concentration of the spray solution used, and affects the estimation of the toxicity of leaf sprays to annuals, while the detoxicating effect of the soil is of primary importance in determining the efficacy of herbicides for perennials.

This investigation had as its object the development of a suitable method for estimating the inherent toxicity of chemicals to plants, and its application to certain substances already studied by other methods. Certain results obtained in the early stages of this study indicated that the relative growth rate of the plants might be a better criterion of toxicity than mortality. The relation between growth rate and dosage was therefore investigated.

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The observed effect of the chemical on the plant is doubtless determined by the concentration of the substance within the plant, although the dosage is generally expressed in terms of the quantity and concentration of the toxic solution applied as a spray, or the concentration of the toxic chemical present in the culture solution. The rate and extent to which different chemicals, or different concentrations of the same chemical, are absorbed, will therefore be a factor influencing the efficacy of a given treatment. An extensive study of this subject was not contemplated, but a number of analyses of the culture solutions to which various chlorates were added permitted an estimate to be made of the extent to which these substances were absorbed.

Materials and Methods

In order to avoid the complications introduced when the toxic solution is applied as a spray, the test plants were grown in culture solution to which different quantities of the chemicals to be tested were added after the plants had developed. This enabled the dosage to be expressed in terms of the concentration of toxic material in the growth medium. It is well known (2, 3, 9) that apparently identical individuals vary considerably in their susceptibility to toxic substances and it seems reasonable to believe that the susceptibility of unselected wild plants such as weeds will be subject to even greater variability. The variability from this source was reduced as far as possible, by using Marquis wheat, grown from carefully selected seed, as the test plant.

The culture solution used consisted of 5 ml. molar calcium nitrate, 5 ml. molar potassium nitrate, 2 ml. molar magnesium sulphate, and 1 ml. molar potassium dihydrogen phosphate, per litre of distilled water. To this solution 1 ml. of 0.5% ferric tartrate was added initially, and every third day thereafter during the initial growth period, and every second day during the final observational period.

The six-week period from germination until the final observation was made may be divided into three phases: germination, initial growth period, and the observational period following treatment. The germination phase consisted of soaking the seeds for 16 hours in culture solution and then placing them on blotters or sawdust soaked with culture solution. These proved to be the best of the several sub-strata for germination tested at the time. The trays containing the seeds and the substratum were then placed in a chamber, covered with a glass plate to provide a high relative humidity, in the greenhouse in which the subsequent tests were made. The temperature was not controlled. Light was excluded for the first few days, and the seedlings were protected from direct sunlight for the remainder of the germination period, which was six days.

At the end of this period the seedlings were selected for size, and transplanted into the experimental jars. These consisted of two-quart sealers previously painted with one coat of black enamel to exclude light, and one coat of white paint to reduce heat absorption. These jars were fitted with

flat corks provided with five holes into which the seeds were secured with cotton batting. The tare weights of the jar and the cork and cotton batting were determined previously so that the weight of the plants and culture solution could be computed at any time from subsequent weighings. In selecting the seedlings, the length of the sprout was the only index of size that could be used without risk of injuring the seedling. All plants within $\pm 5\%$ of the mean height had to be used, as only about 20% of the seedlings fell within these limits.

During the initial growth period of 14 days the weight of the jar and culture solution and the weight of the cork and the plants were determined every three days. The water lost by evaporation and transpiration was made up with distilled water after each weighing. For weighing the plants and corks, a sheet metal guard, having an inverted-funnel shape, was placed on a torsion balance and the cork inserted in the small end. This guard provided a space, at a high relative humidity, for the hanging roots. A drain pan, which did not rest on the balance, was arranged to catch the drip from the roots. Drainage was complete, within the sensitivity limits of the balance, in less than two minutes and the roots did not appear to suffer from exposure to the atmosphere for these short periods.

The observational period extended from the 14th to the 35th day after planting in the jars. On the 14th day, freshly prepared nutrient solution was placed in all the jars to ensure against any deficit in the elements necessary for growth, and the required dosage of the toxic chemical under test was added to the treated jars at the same time. The jars and plants were weighed, as previously described, every two days, and the water loss made up to keep the concentration of the toxic chemical reasonably constant. The final observations on the living plants were made on the 35th day, or 21 days after the addition of the toxic solution. Treatments that resulted in complete mortality within this period were terminated when the plants died. The final observations included separate determinations of the wet weights of the entire plant, all leaf tissue, living leaves, dead leaves, and all roots from each jar. As the wet weight is subject to considerable variation due to variable temperature and humidity conditions, the oven-dry weights of these fractions were also determined for the first few series of treatments. It was then found that the final weight of the plant was a much more variable quantity than the growth rate and, since this quantity had to be computed from the wet weight, further determinations of the oven-dry weights were abandoned.

All the plants were grown in an ordinary greenhouse, subject to the usual variations in growth conditions, during the summer season, *i.e.*, May to September, inclusive. Eight series of plantings were made at different times and these were consequently subject to slightly different growth conditions, as evidenced by the different growth rates subsequently reported. Unfortunately the second series of plants was not as uniform in size as the others at the time of planting and was subsequently affected by a slight

infestation of aphids in the greenhouse, so that the results of only seven series are reported. The dosages tested included 0.005, 0.010, 0.015, 0.025, 0.050, 0.075, 0.125, 0.175, 0.250, and 0.500% of the culture solution on a weight basis. All of these dosages were not used with all chemicals, and a few intermediate doses were employed in some cases. All treatments were made in duplicate on plants grown at the same time, and in a few instances certain treatments were repeated in subsequent series to obtain an estimate of the variation between series. From 6 to 10 untreated controls were used with each series of plants.

The analyses of the chlorate solutions were made by reducing the chlorate with an excess of tenth normal ferrous ammonium sulphate solution, and titrating the excess with standard potassium dichromate solution, using diphenylamine as an internal indicator. The ferrous ammonium sulphate was standardized against the potassium dichromate solution before each lot of analyses. In making the determinations the air was removed from the flasks with carbon dioxide, the ferrous ammonium sulphate, and chlorate, solutions added and boiled for 10 min. After cooling and dilution, 15 ml. of an acid mixture, required to provide the conditions necessary for the reaction and titration, was added. This mixture consisted of 150 ml. of syrupy phosphoric acid and 150 ml. of concentrated sulphuric acid in a litre of solution. Three drops of the indicator were then added and the solution titrated. This method was found to be quick and accurate, and has a sharp end point which is not affected by the presence of small quantities of organic matter, as is the permanganate titration.

Estimates of Relative Toxicity from the Certainly Lethal Dose

It was expected that the dosage-mortality results obtained would be capable of being treated by the methods described by Trevan (9) and Bliss (2, 3) and thus allow the effective dosage, and the extent to which it might be in error, to be evaluated. When the experiments were made, however, it was found that, if a given dosage produced complete mortality, the next lower dosage usually killed none of the plants within the test period, although it reduced the growth considerably. Under these conditions the certainly lethal dose (C.L.D.) was the only estimate of the relative toxicity obtained, and precise estimates of the extent to which this dosage might be in error were impossible. Duplicate treatments made on plants grown at the same time usually gave the same mortalities, but some variability was evident between series. Arbitrary estimates of the variability are unsatisfactory, and since the chemicals had a decided effect on the size of the plant at dosages that produced no mortality, it appeared that the weight, rather than the mortality, might give a better estimate of the effect of the chemical, and so no attempt was made to determine the dosage-mortality relation more precisely.

In those cases in which the certainly lethal dose of the chemical fell within the dosage range studied, the value obtained is reported in Table I, the chemicals being arranged in order of decreasing toxicity. The relative toxicity

TABLE I

RELATIVE TOXICITY OF CHEMICALS ADDED TO CULTURE SOLUTION, AND APPLIED AS
A SPRAY TO PLANTS GROWN ON SOIL

Chemical	C.L.D. in culture soln., %	Relative toxicity when applied as a spray to plants grown on soil	
		Annuals Conc. of spray soln. = 10%	Perennials Conc. of spray soln. = 25 to 20%
Sodium cyanide	0.005	I	III
Sodium arsenite	0.005	II	II
Sodium dichromate	0.010	I	II
Arsenic pentoxide	0.010	I	III
Ammonium thiocyanate	0.015	I	II
Sodium selenite	0.015	I	II
Calcium hypochlorite	0.025	V	—
Nickel sulphate	0.050	VII	—
Sodium arsenate	0.050	IV	—
Sodium sulphide	0.050	III	IV
Potassium permanganate	0.062	VIII	—
Zinc chloride	0.075	II	—
Sodium ferrocyanide	0.075	IV	—
Ammonium chlorate	0.125	II	—
Copper nitrate	0.125	II	IV
Copper sulphate	0.125	V	—
Calcium chlorate	0.250	IV	—
Sodium chlorate	0.250*	II	I
Sodium perchlorate	0.250	V	—
Lead nitrate	0.250	VIII	—
Aluminium sulphate	>0.250	VIII	
Zinc sulphate	>0.175	V	
Calcium chloride	>0.500	VI	
Potassium chloride	>0.500	VII	

* 0.200% gave complete mortality, but as this dosage was not used with the other chemicals, the higher, comparable, value is reported.

of some of these chemicals, when applied as a spray to annual and perennial weeds, has already been estimated from the C.L.D. under these conditions and reported in earlier publications (5, 7, 8). The grouping of these substances on the basis of their relative toxicity is also given in Table I for comparative purposes, the groups bearing the lower numbers being the more toxic. For annual weeds, substances falling in Groups VII and VIII, and for perennial weeds, those falling in Groups III and IV, are not sufficiently toxic to be of any value as herbicides.

It is evident from the results that the inherent toxicity of a substance, as judged from the culture solution tests, gives no indication of its value as a herbicide for perennials. This is in agreement with earlier results (7, 8)

which showed the efficacy of a chemical for killing perennials to depend on the interactions that take place between the chemical and the soil, as well as on its inherent toxicity.

There is somewhat better agreement between the results obtained in culture solution and those obtained from the spraying tests on annuals. Of the five chemicals found to be most toxic in culture solution, four were classified in Group I and one in Group II in the spraying tests. The remaining less toxic substances are placed in a different order of relative toxicity by the two methods, certain substances being comparatively more toxic in culture solution than as a spray and *vice versa*. Some of these discrepancies can doubtless be attributed to the experimental errors applicable to the estimated C.L.D. by both methods. On the other hand, the two methods differed in that the dosage in culture solution was varied by adjusting the concentration of the toxic substance, whereas in the spraying tests it was varied by altering the volume of a spray of fixed concentration. Where the spraying test showed a given substance to be relatively less toxic than was indicated by the culture solution test, the results can be explained by the discrepancy between the dosage applied as a spray and that retained by the plant, as the divergence between the applied and retained quantities increases (6) as the volume of spray containing unit quantity of toxic chemical increases. Where the culture solution method indicates a lower relative toxicity than the spraying test, the explanation probably lies in the lower concentration of the chemical in the culture solution than in the spray. The concentration of the solution doubtless determines the amount of poison absorbed by the plant (see p. 536) and may also determine its mode of action, *e.g.*, destruction of individual cells or tissues, or transport through the plant.

Although the results of toxicity tests in culture solution are not highly correlated with those obtained in spraying tests, they might be useful when used in conjunction with field tests. In the first place the culture solution determinations can be made more quickly and easily than field trials, and could be used to eliminate substances of low inherent toxicity. Secondly, if the minimum volume of spray required for coverage under field conditions were known, the relative values of the C.L.D. of different chemicals in culture solution might be useful for estimating the best concentration of each chemical to be applied in field tests.

Time Required for Death of Plant Following Treatment

The time required between treatment and death of the plant is important from both the experimental and practical standpoints. Experimentally, the effect of a chemical is determined after a limited observation period, and slow-acting substances may appear to be less effective than those that act more rapidly. Under field conditions a slow-acting poison may permit considerable growth to occur following its application, and this not only complicates the interpretation of the results, but lessens the value of the treatment, as compared with substances that act quickly.

It was hoped in this investigation to obtain a reasonably reliable estimate of the rate at which the different chemicals acted. When the experiments were made it was found impossible to obtain any precise estimate of the time required, owing to the difficulty of determining precisely when the plant was dead. Some plants that appeared to be dead revived when transferred to culture solution free from toxic substances, while others, treated in other ways and apparently alive, died after such a transfer. Nevertheless some of the general features of the results are of interest.

When the elapsed time between treatment and the estimated death point was plotted against the dosage in terms of concentration, the position of the curves for the various chemicals was determined largely by the C.L.D. of the particular chemical and the time required for death at this dosage. Where sufficient data were available, however, it was evident that the curves for all chemicals were of the same general form. The time required for death at first decreased as the dosage increased, and then flattened off at higher dosages, approaching a constant value apparently independent of the dosage. The dosage range employed made it possible to determine this limiting time for 10 of the chemicals. These were as follows: arsenic pentoxide, 4 days; sodium arsenite and sodium selenite, 5 days; copper nitrate and sulphate, 6 days; sodium dichromate, sodium arsenate, and calcium hypochlorite, 8 days; and ammonium thiocyanate and sodium cyanide, 10 days. These limiting times, being independent of the dosage, must be determined either by the rate of absorption of the chemical, or by the rate of the reaction producing mortality within the plant. Comparison of the times given above with the toxicity of the chemicals as judged from the C.L.D.'s reported in Table I indicates that the time required to cause death is independent of the toxicity of the compound.

The dosage-time relation was then studied after reducing these two quantities to a comparable basis for all chemicals. This was done by converting the dosage, in terms of concentration, to the number of C.L.D.'s it represented, and the time in days to a percentage of the time required at the C.L.D. When these quantities were plotted all the curves fell into two groups, the curves for each of the chemicals in the two groups coinciding over the dosage range up to about 2 C.L.D. Beyond that point the curves flattened out at various levels corresponding to the fixed times already reported. The group having the greatest initial slope, and the greatest final reduction in killing time, included only arsenic pentoxide and sodium selenite. All the other chemicals for which adequate data were available fell into the second group. The difference between these two sets of curves, on a comparable physiological basis, supports the earlier conclusion that the rate at which a chemical acts is independent of its inherent toxicity.

Dosage-Weight Relations

It has already been pointed out that sub-lethal doses seldom produced partial mortalities, although they reduced the weight of the plants considerably. The use of shorter dosage intervals might have produced partial

mortalities over a short range, but estimates of the toxicity based on this criterion would have neglected the toxic effect of the chemical as indicated by the reduction of weight at low dosages. The extent to which the wet weight is reduced by sub-lethal doses is shown by the results given in Table II for three of the chemicals. It appeared from these results that the methods and analysis described by Trevan (9) and Bliss (2, 3) applicable to the dosage-mortality relation might actually be applicable to the dosage-weight relation in plants. The use of the weight, rather than the number, of living plants has already been suggested (7), on the basis of practical considerations, for determining the efficiency of herbicides in the field, and other investigators (4) have used the weight of the plants as a criterion of toxicity.

TABLE II
DOSAGE-WEIGHT RELATION

Dosage conc. in culture soln., %	Wet weight of entire plant as percentage of corresponding controls		
	Sodium chlorate	Zinc sulphate	Aluminium sulphate
0.025	62.8	58.2	50.6
0.050	31.2	42.8	21.8
0.075	25.8	28.5	20.3
0.125	14.7	17.3	21.8
0.150	23.1*	—	—
0.175	20.0*	22.8*	18.8
C.L.D.	0.200%	> 0.175%	> 0.250%

* Results from a later series under slightly different growth conditions.

In view of these facts an attempt was made to determine the relation between the dosage and the final weight of the plants. It was evident that the final weight of the entire plant, on either a wet or dry basis, could not yield a sigmoid curve when plotted against dosage, since the final weight of the plant tissue, whether dead or alive, would always have a finite value, dependent on the size of the plant used. The weight of living tissue at the end of the experiment was therefore used in an attempt to establish the relation, but in spite of the large body of data available, the results were too variable to permit a definite conclusion. Part of the observed variability doubtless arose from the rather arbitrary separation of the living and dead tissue. The difficulty of determining when an entire plant is dead has already been mentioned, and this problem is present in greater degree in tissue separations. However, the greater part of the variability was contributed from other sources; namely, the final wet weight of the entire plant, whether treated or not, and this variability was reflected in the weights of the living and dead tissues on both a wet and dry basis. Since this variability could not be attributed to a variation in the susceptibility of the plants to the chemicals, the untreated controls being subject to fluctuations of comparable magnitude, a further study of the dosage-weight relations was abandoned in favor of a study of the dosage-growth-rate relations.

Relative Growth Rate as a Criterion of Toxicity

The relative growth rate, or the increase in weight per unit of weight already attained, as well as per unit of time, takes into account the variable initial weight of the plant, and the rate at which the chemical acts. Making use of the equation $\frac{1}{w} \frac{dw}{dt} = \frac{d \log w}{dt}$, the relative growth rate was computed from the natural logarithms of successive weights. When this was done, it was found that the coefficient of variability of the growth rate was much smaller than that of the final weights. For instance the means and standard errors for the plants in the 10 untreated control jars in the first series were: final wet weight per jar, 68.6 ± 8.3 gm. and the mean relative growth rate over the entire period, $8.90 \pm 0.33\%$ per day. These values give coefficients of variability of 12.1% and 3.7% respectively. The relative growth rates for both treated and untreated jars in all series were similarly less variable than the final weights.

The weights of the plants during the first two-week growth period were so small that the growth rate could not be determined accurately, and the weight on the 14th day, when the poison was added, was taken as the initial value. In consequence it was impossible to determine whether the weight differences then observed were the result of slight differences in size that were not detected when the selections were made, or were caused by slight injuries to the seedlings at the time of planting which affected their growth rate during the initial growth period.

Before considering the relation between relative growth rate and dosage, the factors affecting the comparability and variability of the growth rate over the observational period, *i.e.*, 14 to 35 days, must be examined. During this period the plants were weighed every two or three days, which permitted the relative growth rates to be calculated independently for each of these periods. In Fig. I, A the observed growth rates have been plotted against the midpoint of the period over which they were observed, for the untreated controls in the seven series. It is evident that the growth rate varies between series, and within series it varies from day to day, a result attributable to the different growth conditions prevailing at different times. The curves also give some indication of the magnitude of the reduction in growth rate with age.

The variation in growth rate between series shows that valid comparisons of the effect of different treatments cannot be made on plants grown at different times until the effect of the chemicals on plants having different growth rates has been established. Even within series the variation in the growth rates from day to day may introduce a disturbing influence if the treated plants respond to the variable conditions differently than the untreated controls. This possibility was tested by plotting the growth rates of treated and untreated plants in the same series against time. A typical graph of this sort, for the results obtained with sodium chlorate, is shown in Fig. I, B. It is evident that although the mean relative growth rate decreases as the

dosage of poison increases, the growth rate of the treated plants from day to day varies, in most cases, in accordance with the growth rate of the untreated controls. It is of interest to note that not only was this true for treated plants that showed a positive growth rate, but also for the maximum dosage when

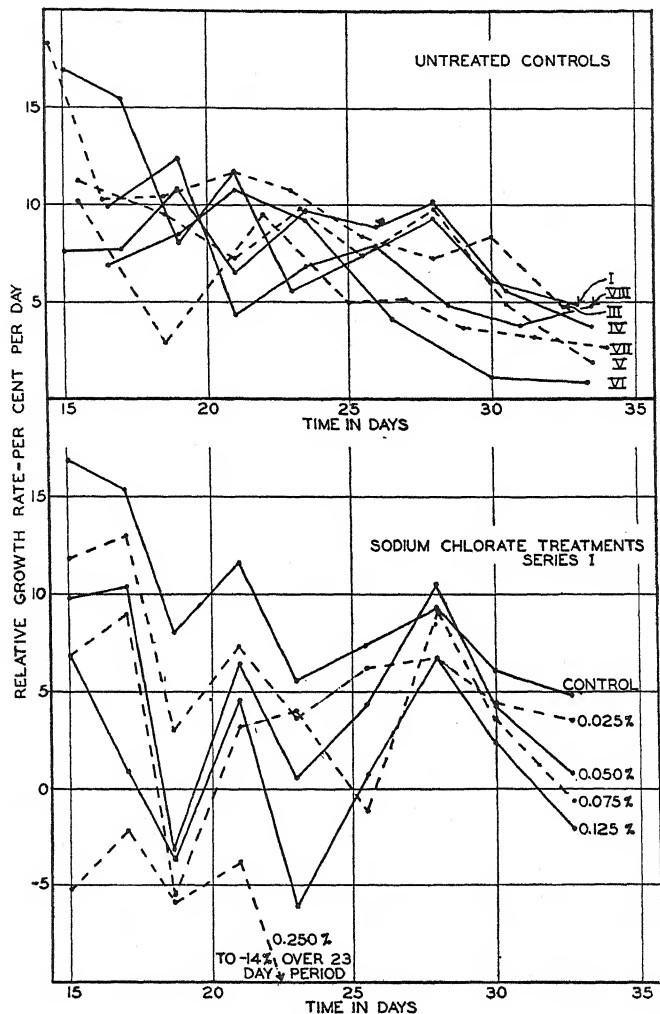


FIG. 1. Growth rate of untreated and treated plants during observation period.

the plants had a negative growth rate throughout. The parallel behavior at negative growth rates may be fortuitous, since the small initial weight decreased with time, and the errors in weighing such small quantities of material could easily account for the observed differences.

The steady decrease in growth rate with age of plant did not appear to be a serious complication. If a given treatment did not cause complete mortality at the end of the observation period, the average rates for the treated and

untreated plants would be comparable. Certain treatments, however, killed the plants a few days after the toxic chemical had been added, and in such instances the final weighing was made as soon as the observer was sure that the plants were dead. Here, the comparable value of the growth rate for the control would be that observed over the period between treatment and death of the treated plant, rather than the average over the entire observational period. The difference between the growth rate of the controls over the two periods in question was subsequently found to be small compared with the variability arising from other sources.

The practice of expressing the loss of weight associated with death as a negative relative growth rate may be questioned. The decrease in weight is due largely to the loss of moisture from the dead tissue, and since its magnitude will depend on the weight of the plant as well as on the time, the use of a relative rate appears to be sound. On the other hand it must be recognized that the relative rate of moisture loss from dead tissue may differ considerably from the relative rate of growth of living tissue under similar conditions. Since the plant tissue will dry out continuously, and the death-point of the plant cannot be determined precisely, it is evident that the negative growth rates associated with death will be subject to considerable variability.

Before proceeding to a discussion of the average growth rates over the entire observational period, it is of interest to consider the systematic changes in growth within the observational period for treated plants. In order to do this the growth rates between the initial and succeeding observations were computed and plotted. This procedure "smoothed" the curves and eliminated most of the erratic day-to-day variations. It was then found that treatments with slow-acting poisons, such as the chlorates, behaved somewhat like the untreated controls, in that the growth rate decreased during the first week of the observational period and then remained at a more or less constant value. As the dosage of such chemicals was increased, the curve was changed in position to lower growth rates but retained the same general shape. A dose of 0.2% sodium chlorate caused a continuous loss of weight corresponding to a negative growth rate of about -1% per day throughout the entire period from the second to the seventeenth day after treatment, when the plants were dead. On the other hand plants treated with quick-acting chemicals, such as the arsenicals, suffered weight losses corresponding to continuously decreasing growth rates. Thus 0.01% of arsenic pentoxide decreased the growth rate from 7.0 to 5.0% per day during the first 2-day period while the value over a 12-day period was -4.7% per day. The other chemicals usually showed a behavior between these two extremes, but a few showed evidence of stimulation during the first two or three days that was not evident over longer periods. Some treatments caused a continuously decreasing growth rate, but the plants were not dead at the end of the observational period. Chemicals exhibiting this behavior might have caused complete mortality over a longer period of time, at lower dosages than those reported in Table I.

The average growth rate over the observational period or, when the plants died within this time, over the period between treatment and death of the plants, was used to plot growth-rate-dosage curves. In order that the reported growth rate would not be based entirely on the initial and final weights, the growth rate was computed for both the period between the first and last, and the second and second-last weighings. These two values were then averaged to obtain the growth rate for each jar, and the replications of any one treatment, in any one series, were again averaged to obtain the reported values.

The curves obtained are shown in Figs. 2 and 3, the dosage being expressed in terms of per cent concentration of chemical in the culture solution. Within the dosage range studied, there was no evidence of stimulation over the entire period, all the treatments giving growth rates comparable with, or less than, the untreated controls. The curves are generally quite smooth throughout the range of positive, and for a short range of negative, growth periods. Large negative growth rates frequently showed considerable variability and many of the points obtained at dosages in excess of that required for complete mortality have been omitted from the graphs for the sake of clarity. The curves are usually slightly concave upwards, but sometimes tend to flatten off abruptly at large negative growth rates.

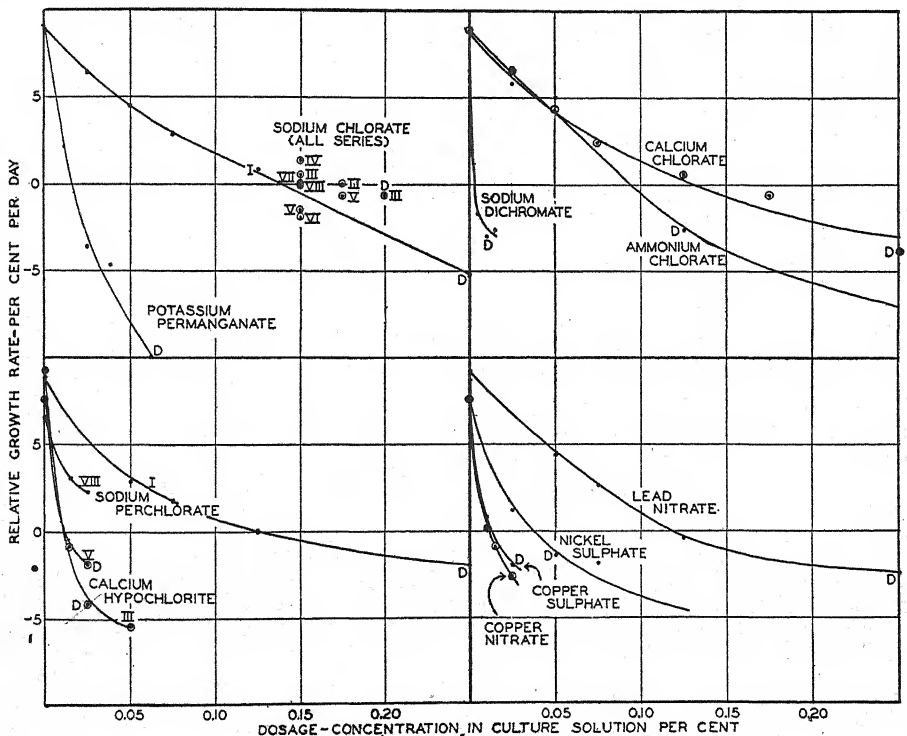


FIG. 2. Relation between growth rate and dosage.

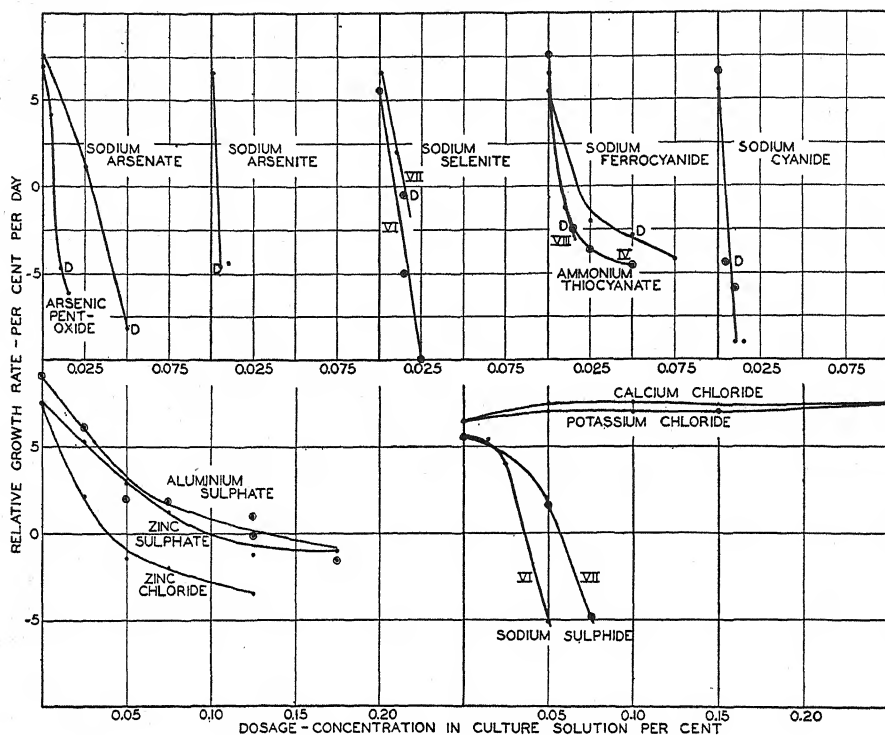


FIG. 3. Relation between growth rate and dosage.

Analysis of the dosage-mortality relation (2) indicates that, in many organisms, the effect of the toxic substance increases in proportion to the logarithm of the dosage rather than the dosage itself. It might be expected therefore that a linear relation would exist between the logarithm of the dosage and the relative growth rate. The available data were inadequate to permit this relation to be tested for all the chemicals used, but the results for six substances are plotted on this scale in Fig. 4. It appears from these graphs that the relation is a linear one within experimental error. This result is in agreement with Bateman's findings (1) which show a linear relation between the logarithm of the percentage retardation in growth and the logarithm of the dosage, for a number of organisms. There appears to be no advantage in Bateman's method of expressing the growth obtained with added poison as a percentage of the controls, or avoiding the inverse relation by computing and plotting the retardation instead of the observed growth rate, against the dosage. It appears from Fig. 4 that the position and slope of the lines, for a given species grown under the same conditions, are determined by the properties of the chemical.

It is obvious that treatments that reduce the growth rate compared with that of the controls, will reduce the relative size of the plants, although these growth rates may be positive. Partial mortalities were seldom observed in

these experiments, but the few that did occur fell in the region of positive, but retarded, growth rates. In one instance, a 20% mortality occurred at an average growth rate of 2.5% per day. This was the lowest partial mortality and the largest growth rate at which any plants died in these experiments.

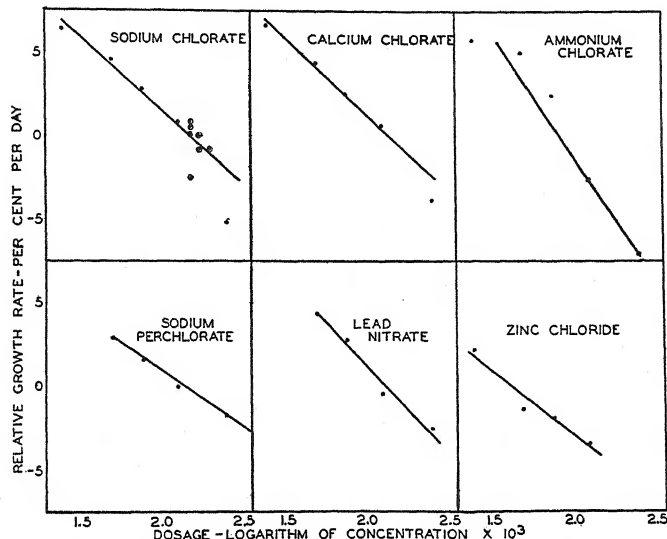


FIG. 4. Relation between growth rate and the logarithm of the dosage.

The next point was to determine the growth rate at which complete mortality occurred. On theoretical grounds it would appear that a growth rate of zero should result in complete mortality if the observations were made over a sufficient period of time. In practice, however, the death of the plant cannot be detected until some time after it has occurred. The continuous loss of weight during the interval between the actual and observed death points introduces a bias which brings the observed growth rate into the negative region, and this bias tends to be greater as the time between treatment and death decreases.

In order to determine the growth rate corresponding to complete mortality, the growth rates at the C.L.D. for all chemicals, *i.e.*, the points marked D on the curves in Figs. 2 and 3, were treated statistically. In making these analyses, the results obtained with potassium permanganate and the arsenicals were excluded, since the C.L.D.'s occurred at comparatively high negative growth rates. It was observed that potassium permanganate affected only the roots actually immersed in the solution, so that the plant probably died from mineral starvation rather than from a toxic effect. The high negative growth rates observed at the C.L.D. of the arsenicals are probably the result of inadequate data. Excluding these substances, and those whose C.L.D. did not fall within the dosage range studied, the mean growth rate for all the other chemicals at the C.L.D. was found to be -2.44% per day.

The individual values fluctuate around this mean with a standard deviation of $\pm 1.35\%$ per day, but the variance included in this standard deviation is contributed from three distinct sources: the variance between duplicates; the variance between series, since the different chemicals grouped in the above calculation were tested at different times; and the variance between chemicals, if different poisons cause complete mortality at different growth rates. Unfortunately the data were inadequate for a complete analysis of the variance contributed by these three sources, but it was possible to make some estimate of the standard error of duplicates and the variance between series.

In computing the standard error of duplicates the complete results were divided into four groups: (1) controls; (2) treated plants with growth rates above 0% per day but below controls, *i.e.*, in the subnormal growth and partial mortality region; (3) treated plants with growth rates between 0 and -5% per day, *i.e.*, in the range in which complete mortality can be expected; and (4) growth rates less than -5% per day. The values obtained are reported in Table III, from which it is evident that as the growth rate decreases

TABLE III
GROWTH RATE: STANDARD ERROR OF DUPLICATES

Range of growth rates	Degrees of freedom	Standard error, % per day
Untreated controls	43	0.46
Treated plants, growth rates greater than 0% per day, <i>i.e.</i> , sub-normal growth and partial mortality range	51	0.60
Treated plants, growth rates 0 to -5% per day, inclusive, <i>i.e.</i> , complete mortality range	55	0.86
Treated plants, growth rates less than -5% per day, <i>i.e.</i> , complete mortality range but dosage excessive	35	1.16

the standard error of duplicates increases. This result can be partly explained by the fact that the percentage error of weighing increases as the weight decreases, *i.e.*, as the growth rate decreases. It seems likely, however, that the error in the negative growth-rate regions associated with death is also affected by the difficulty of estimating the exact death point of the plants.

The untreated controls differed significantly between series. This, however, is of less interest than the variance between identical treatments in different series. A few tests of this type, corresponding to 19 degrees of freedom, resulted in growth rates between 0 and -5% per day, the range of greatest interest. Analysis of these data showed that the variance between series and chemicals was significantly greater than the variance between the corresponding parallel treatments. Further analysis indicated that different

growth conditions, as indicated by the relative growth rate of the controls, affected certain chemicals in one direction and others in the reverse way, but the data were too few to permit a definite statement. The effect of similar treatments on plants having different growth rates is shown in Figs. 2 and 3, by the points obtained with sodium chlorate in all series, and the curves obtained in different series for some of the chemicals. It is therefore evident that the standard error of duplicates is only a small part of the error involved in estimating the relative toxicity of two chemicals, particularly if these determinations are made at different times.

In conclusion it appears that the relative growth rate is a more useful measure of the effect of chemicals on plants than mortality. Where chemical treatments capable of destroying the weeds entirely are too costly, it may be practicable to apply dosages that will control the weed by reducing the growth rate. Here the relative toxicity of different chemicals should be assessed from the dosage range that causes a reduction in growth rather than from the dosages required to produce complete mortality. Since the relation between relative growth rate and the logarithm of the dosage appears to be linear, over the range of practical interest, the relative toxicity of different chemicals can be readily estimated from the dosages required to produce any given growth rate.

The relative growth rate can also be used for predicting the dosage required to cause complete mortality. It seems likely that this method, when calibrated, would be less time-consuming than determining the mortality directly. When the latter method is used, a considerable period must elapse between treatment and the final observation to ensure that the maximum mortality is obtained, and to ascertain whether or not the affected plants are dead. The error of predicting the mortality from the growth rate appears to arise mainly from the variance between chemicals and between test plants grown at different times, *i.e.*, under different growth conditions, the standard error of duplicates being relatively small. The variable behavior of plants grown at different times is also common to toxicity determinations based on mortality. On the other hand the growth rate method permits a study of the interactions between chemicals and growth conditions, as indicated by the growth rate of the untreated plants, thus providing information as to the possible effect of variable environmental conditions on the efficacy of a chemical.

Absorption of Chlorates from Culture Solutions

Chlorate salts form the basis of many commercial herbicides. Previous investigations (4, 5, 6, 7, 8) have shown that they are quite toxic, and since they are not readily detoxicated in the soil they are effective for the destruction of perennials. It is evident, however, from results presented in this and earlier papers that they act rather slowly as compared with several other chemicals. These considerations led to a few preliminary measurements on the amount of three chlorate salts absorbed from the culture solution at sub-lethal dosages. No analyses are reported on solutions containing lethal

dosages, since the exact time at which the plant died could not be judged accurately, and contact with the dead roots might decompose part of the chlorate salts and yield fictitiously high results for absorption.

The initial concentration of chlorate salts was known from the analysis of the stock solution and the dosage used. No determinations were made during the observational period. Before determining the final chlorate content of the solutions, sufficient distilled water was added to bring the weight up to the original value. Duplicate analyses were made on the solution from each jar, and the results reported in Table IV are based on the

TABLE IV
ABSORPTION OF CHLORATES FROM CULTURE SOLUTION

Chemical	Amount added per jar, gm.	Amount absorbed		Ratio of amount absorbed to final wet weight of plants, %	Ratio of conc. absorbed to conc. in culture soln., %
		gm.	%		
Ammonium chlorate	0.50	0.06	12	0.20	8
Ammonium chlorate	1.00	0.12	12	0.47	9
Ammonium chlorate	1.50	0.06	4	0.46	4
Sodium chlorate	0.50	0.09	18	0.44	10
Sodium chlorate	1.00	0.10	10	0.45	10
Sodium chlorate	1.50	0.16	10	0.90	12
Sodium chlorate	2.50	0.19	8	2.33	20
Calcium chlorate on anhydrous basis	0.43	0.08	18	0.26	16
Calcium chlorate on anhydrous basis	0.87	0.08	9	0.35	10
Calcium chlorate on anhydrous basis	1.30	0.11	8	0.74	11
Calcium chlorate on anhydrous basis	2.17	0.10	5	1.04	10

average of four determinations at each dosage. The duplicate samples taken from the same jar showed close agreement, indicating that the method was satisfactory, but there was frequently considerable variability between the duplicate jars treated in the same way, and this source of variability probably accounts for most of the evident irregularities. In general, the amount of chlorate absorbed increased with the dosage or initial concentration, but on a percentage basis less was absorbed from the more concentrated solutions. Since the weight of the plants decreased as the chlorate concentration increased, the quantity absorbed was expressed as a percentage of the wet weight of the plant. These figures, given in the fifth column of Table IV, are more regular than the others and show the importance of considering the size of the plant in determinations of this sort. In order to obtain an estimate of the effective toxic concentration within the plant, the percentage of chlorate

salt in the plant was plotted against the growth rate, and extrapolated to the growth rate corresponding to 100% mortality. This indicated that a chlorate salt concentration of about 3 to 4% of the wet weight would be required to produce complete mortality. The data were too few to permit a definite conclusion, but the above figure is in agreement with some of the lower values computed from analysis of solutions containing lethal dosages in which the chlorate is subject to decomposition as mentioned previously.

The amount of chlorate salt absorbed and the total amount of water transpired during the observation period was used to calculate the average chlorate salt content of the solution absorbed by the plant. This concentration, expressed as a percentage of the concentration present in the culture solution, appears in the last column of Table IV. These results show that, on the average, the plant absorbed a solution containing only one-tenth as much chlorate as that present in the culture solution. Some of the data obtained suggested that this marked selectivity on the part of the plant may break down as the death point is approached, but this cannot be stated with assurance. This highly selective absorption of chlorates is nevertheless extremely important. In the first place it shows that chlorates must be added considerably in excess of the amount required to kill the plant. Secondly, it shows the effect of dilution on the efficacy of chemicals of this type. It has already been shown (7) that the concentration and quantity of the solutions normally used against perennials in the field act mainly through the soil. Since the free moisture content of soil is subject to great variation, it follows that the efficacy of a given dosage will be subject to large variability from dilution, quite apart from differential losses by detoxication and leaching.

Acknowledgment

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PHYSIOLOGIC CURVE OF RESPONSE TO PHYTOHORMONES BY SEEDS, GROWING PLANTS, CUTTINGS, AND LOWER PLANT FORMS¹

By N. H. GRACE²

Abstract

In all plant species tested, increasing concentrations of phytohormones produced responses falling on a physiologic curve from minimum through optimum to maximum which, if exceeded, led to injury and death. Indolyl-acetic acid, its butyric and propionic homologues, naphthylacetic acid, their salts, and mixtures gave similar results. Treating seeds with hormones incorporated in adsorbent dust stimulated both root and top growth markedly, with less danger of overdosage than in solution treatment. Dosages equivalent to 50 to 250 mg. per acre applied as dilute solutions to soil growing young lettuce and tomato plants covered the optimum range of stimulation to growth. Dust treatment of cuttings proved very convenient and successful in inducing rooting, the plants again showing a wider range of tolerance to dusts than to solutions. Fermentation of sugar by yeast responded to hormone stimulation. Various practical applications are discussed.

Introduction

The physiological activity of the group of chemicals loosely designated by the general term plant growth substances or phytohormones has been established through the work of Kögl, Went, Thimann, Laibach, Zimmerman, Hitchcock and many others. Recent study of the practical utility of these compounds has been related to their ability to cause and hasten root formation by plant cuttings. The results of this work have led to their successful use in plant propagation. While the epinastic or bending response is widely used as a criterion of physiological activity, the magnitude of this response does not give a comparative measure of the root-producing power of the various compounds (7). Among the other responses reported recently are those relating to the effect of heteroauxins on legume nodule formation, and the production of parthenocarpic fruits (3, 5).

It is commonly stated that growth hormones do not increase root development of growing plants; in fact many investigators report an inhibition of root growth. The present writer has found such inhibition to be due usually to overdosage. Plant roots are extremely sensitive even to low concentrations of the active chemical; ordinarily dilute solutions may exert a damaging effect. However, if an exceedingly small amount of phytohormone is applied to the germinating seed or to the plant in a gradual manner, pronounced growth stimulation is usually noted. As hormone dosage increases, growth stimulation also increases until a peak is reached, then falls off to zero and finally below the value for untreated control plants. The results when plotted describe the familiar physiological curve. Such results have been obtained with 3-indolylacetic acid, its homologues, γ -(3-indolyl)-butyric and β -(3-

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indolyl)-propionic acids, and with 1-naphthylacetic acid, and mixtures and salts of these applied to seeds of many kinds, to growing plants such as wheat, tomatoes and nasturtiums, and to yeast and certain bacteria.

It must not be inferred that all the active chemicals are equally effective. The response varies not only with the hormone, but also with the method of application, and with the species and stage of development of the plant. Some plants are more easily damaged or killed by a particular hormone. Generally speaking, there appears to be least danger of shock when the naturally occurring hormone, indolylacetic acid, is used to root cuttings, though in many cases superior rooting occurs with the synthetic compounds. The rooting of cuttings over a range of hormone concentrations follows the same general type of physiological curve obtained with germinating seeds and growing plants. Another interesting phenomenon frequently observed, when the cut ends of herbaceous plants are placed in suitable dilute solutions of phytohormones, is a marked swelling or increased turgor.

This paper is introductory. Only illustrative results of a wide range of experiments are given in the following sections. Investigations are still actively under way, and more detailed accounts of the various phases, where desirable, will appear in due course.

Hormone Treatment of Seeds

Many workers in this field have reported inhibition of root development when seeds or very young plants are treated with phytohormones. The writer has found that the usual solution methods of applying the hormones, even in dilute concentration, do retard root growth. However, if the seed is treated in such a manner that a small, gradual supply of active material is made available, there is a resulting increase in both root and top growth. In some cases most striking root development results.

Cholodny (2) has reported a substantial increase in yield when oat seed is soaked in hormone solution prior to planting. We have tried this method with oats and have failed to get any increased yield, using the range of concentrations he described. However, our seed was dried after treatment to conform to recognized seed treatment practice.

The salient feature of the method of seed treatment developed in this laboratory is the use of an adsorbent dust carrier. As the treated seed swells and starts to germinate, the adsorbed, adhering hormone is made available gradually. The dust application of hormone chemicals to seeds provides a ready and accurate method of applying definite amounts. The danger of overdosage is substantially reduced. The carrier may be talc, or other suitable dust, or a standard mercurial dust disinfectant. The growth-promoting chemical is intimately mixed with the carrier by a grinding mix or other thorough mixing operation. The amount of chemical added to the carrier will depend on the dosage desired and the rate of application of dust to the seed. In treating cereal crops one half ounce of dust has been applied to a bushel. Treatment of garden and other seeds has necessitated the use of a

very wide range of dust additions. Hormone treatment is expressed in parts, by weight, of the growth-promoting chemical per million parts, by weight, of seed.

Stimulation of Wheat Roots

As illustrative of the root stimulation that may be obtained by seed treatment, a few values follow. Root measurements were carried out on lots of twenty plants from each group of hormone treatments. The plants were grown in sand at 12° C. and were removed for observation 14 days after planting. Using controls treated with the carrier alone, a mercurial disinfectant dust in this case, treatment with 2 p.p.m. indolylacetic acid resulted in a 65% increase in the length of roots. A mixture of 2 p.p.m. of indolylacetic and 2 p.p.m. of naphthylacetic acids effected an increase of 102% in root length. Treatment with 2 p.p.m. of indolylbutyric acid increased root length by 55%. The dry weights of the tops were increased up to 20%.

Stimulation of Barley Roots

Roots of barley seedlings from dust-treated seed, germinated in sand and washed out 14 days after planting, are shown in Plate I, Fig. 1. The four bunches at the left represent successively the control and three increasing doses of naphthylacetic acid. Photographed with the crowns at the same level, the roots indicate the typical physiological curve obtained in all this work. The lowest concentration of hormone (second bunch) represents the optimum in this case, and the treatment accorded the fourth bunch has obviously exceeded the maximum dosage giving positive stimulation. The fifth bunch (last at right) shows the effect of a mixture of naphthyl- and indolyl-acetic acids, totalling 5 p.p.m., to be positive stimulation falling between that resulting from 2.5 and from 12.5 p.p.m. of naphthylacetic acid alone.

Roots of barley seedlings of the same age and from the same planting, but treated with indolylacetic acid, are shown in Plate I, Fig. 2. The results are of the same general nature though not so sharply defined as those in the preceding experiment. Apparently the plants have a wider range of tolerance to indolylacetic acid, though the response to naphthylacetic acid is often more marked.

In both the foregoing experiments, a standard mercurial disinfectant dust was used as the carrier for the hormones.

Soya Bean Roots

Experiments were made with soya beans grown for six weeks in flats of good soil, using both dust and solution methods of seed treatment. In the latter case the seeds were allowed to dry to a condition suitable for planting in the ordinary way, since the experiments had reference to the agricultural applicability of the method. In Plate I, Fig. 3 the first bunch at the left, dust-treated with 10 p.p.m. naphthylacetic acid, shows a more vigorous response than its next two neighbours, dust-treated respectively with 20

and 5 p.p.m. indolylacetic acid. Of the latter two, the higher concentration proved better. The control bunch at the right was treated only with the standard disinfectant dust used as a carrier in the other three cases.

In Plate I, Fig. 4, the left-hand bunch represents the effect of dust treatment with 10 p.p.m. indolylacetic acid (talc being used as the carrier), compared in the next two bunches with solution treatment at 100 and 10 p.p.m. of solution, respectively. The seed was soaked for an hour, then drained and allowed to dry. Comparison of the two solution treatments with the untreated control at the right, shows that damage has occurred in both cases, whereas the lower concentration applied as a dust (first bunch at left) produced increased growth.

Physiological Activity of Salts of Growth Hormone Acids.

In addition to indolylacetic acid and naphthylacetic acid and combinations, a number of other physiologically active substances have been investigated with seeds. Activity is shown by the propionic and butyric acid homologues of indolylacetic acid and by their salts; phenylacetic acid has also been shown to have a measure of activity. There are indications that the salts are particularly effective growth stimulants when applied by this method. An example of this is shown in Plate I, Fig. 5. Both the naphthylacetic acid and its potassium salt (right) caused increased growth of roots and tops, as compared with the control (left) treated with the disinfectant dust carrier only, but the salt was slightly more effective than the acid.

Dust applications of optimum concentrations of phytohormones to seed have increased the dry weight of the tops of month-old wheat plants, grown in soil, as much as 20 and 30%, and of the roots as much as 65%. The rate of emergence of seminal roots is apparently increased by certain concentrations of growth substance. Overdosage represses both root and top growth. While specific reference has been made to field crops in the above, a number of varieties of garden seeds have also been tested and shown to respond in a similar manner.

Hormone Treatment of Growing Plants

The marked response obtained from seeds dusted with hormones suggested the application of small amounts to growing plants. The earlier reports on direct application of phytohormones to plants have failed to give very definite results. Pearse (6) has studied the effect of phenylacetic and indolylbutyric acids, spraying with 0.1% solutions. Recently Greenfield (4) reported fairly marked growth stimulation from the application of solutions of indolylacetic acid. The writer finds that the use of the correct range of dosage gives very definite and clear-cut results, showing good stimulation with optimum additions, and damage with overdosage.

In Plate I, Fig. 6 shows tomato plants which, eight days after seeding in soil, were transplanted to sand moistened with Hoagland's solution (two plants per four-inch pot, in lots of five pots). Thereafter each pot received

50 cc. daily of Hoagland's solution, to which in the lot represented by the centre pot in Fig. 6, 1/100 p.p.m. and, in the right hand pot, 1 p.p.m. naphthylacetic acid had been added. Additional moisture required was supplied as water. The photograph, taken after 19 days of this treatment, indicates tremendous stimulation at 1/100 p.p.m. and, at this stage, substantial stimulation at 1 p.p.m. Subsequently it became evident that the latter treatment was excessive and injurious. Fig. 7 shows the same control pot beside a pot representative of a lot that had received 50 cc. daily of Hoagland's solution plus 1/20 p.p.m. indolylacetic acid, which also stimulated the plants greatly.

Tomato plants were grown in ordinarily good soil for four weeks, then transplanted to exceptionally rich, heavily manured soil, and treated for 17 days. Representatives of groups grown singly in four-inch pots, shown in Plate II, Fig. 8, illustrate two important points. First, the reaction to added hormones is less than in sand: doubtless the naturally occurring indolylacetic acid is present as a product of decomposition in such soil. Second, the extent of stimulation is greatest in very young plants, falling off as the plants grow older. Similar observations were made in several experiments. Nevertheless, the same general type of physiological curve is nearly always observable. In Plate II, Fig. 8, the optimum probably lies between 1/100 and 1/10 p.p.m. naphthylacetic acid, while 1 p.p.m. (fourth from left) has exceeded the maximum. At a later stage, the plants receiving this concentration showed definite injury, as in the sand cultures.

Nasturtiums, started in soil, transplanted after two weeks to four-inch pots of sand, and treated thereafter as in the foregoing experiments, are shown in Plate II, Fig. 9. This photograph was made after the treatment had continued 23 days. The physiological curve obtained again indicates the optimum between 1/100 and 1/10 p.p.m. naphthylacetic acid, while 2.5 p.p.m. clearly exceeds the maximum tolerance of the plants.

Salvias planted in soil in the usual way, then transplanted to four-inch pots, of soil in this case, were then given one treatment, 100 cc. of naphthylacetic acid solution being added to each pot, the concentration adjusted to give dosages of 10, 5, 2.5, 1.25 and 0.1 mg. of the hormone per pot. Eight weeks later, representatives of the usual groups of five pots were photographed. In Plate II, Fig. 10 the control plant, which received only water, is shown at the left. If it were transposed to its logical position at the right, the usual physiologic curve of response would be evident. At this stage, the dosage of 5 mg. appeared optimum, but five weeks later (Plate II, Fig. 11) the next lower dosage of 2.5 mg. appeared fully equal if not superior to it. The lowest dosage, 0.1 mg. per pot, gave well-proportioned plants, heavier in foliage than the controls, with a deeper green color suggestive of a greater chlorophyll content. The heaviest dosage, while stunting the plants somewhat, induced earlier flowering. Most of these differences are observable in both photographs.

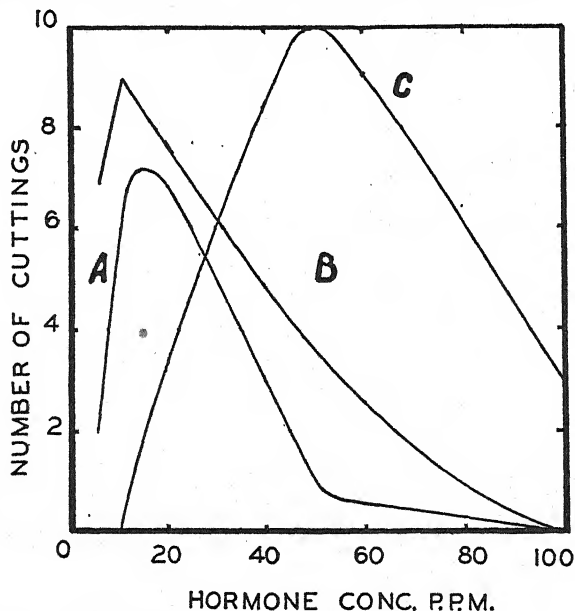
Similar experiments with salvias and petunias, but with the hormone in a dust carrier incorporated in the soil, gave the same general type of results, though less sharply defined.

Lettuce seedlings, growing in soil, were subjected to a more complex experiment, in which the dosages were applied at various concentrations and intervals over progressive periods of time. Plate II, Fig. 12 shows a control flat (left) beside a flat representing approximately optimum effect, this being achieved by 11 treatments at three-day intervals, of 50 cc. 1/100 p.p.m. naphthylacetic acid solution, applied as a fine spray. Solution adhering to the tops was washed down with a water spray. The total amount of hormone applied corresponds to about 120 mg. per acre.

Tomato seedlings were grown and treated similarly. In this case the flats used to illustrate the results (Plate II, Fig. 13) received daily applications of 50 cc. naphthylacetic acid solution, beginning four days after planting. The treatment had continued 16 days when this photograph was taken. The most dilute concentration, 1/100 p.p.m., accumulating a total dosage corresponding to about 200 mg. per acre, gave best results. Solutions of 1 and 5 p.p.m. caused damage, which was very pronounced at 5 p.p.m. Comparing Fig. 13 with Fig. 8, (Plate II), brings out the point already mentioned, that very young plants react more sharply to hormones than do older plants.

Propagation of Cuttings

Experiments with some 4,000 cuttings, representing seven species, have shown dusting with hormones to be a very convenient and successful method of treatment to stimulate rooting. The lower ends of the cuttings, in bunches up to 50 or so at a time, are dipped in the dust, the excess is shaken off, and the cuttings are planted directly. In treatments of this kind the concentration



TEXT-FIG. 1. Rooting response of solution-treated cuttings, using 5, 10, 25, 50 and 100 p.p.m. A: *Salix pentandra* with indolylacetic acid. B: Same with naphthylacetic acid. C: *Weigela rosea* with indolylbutyric acid.

of the hormone is expressed in parts per million of the dust carrier. An example of the results is given in Plate II, Fig. 14, which shows four specimen groups of *Deutzia crenata* after 26 days in sand in a propagating frame, the three beginning at the left having been dusted at planting with indolylbutyric, indolylacetic, and naphthylacetic acids respectively, in a concentration of 1000 p.p.m. talc dust. The effectiveness of the treatments increases in the same order, the vigor of the naphthylacetic-treated cuttings being especially good. The controls, of which specimens are shown at the right, had not rooted.

Comparisons made with the solution method, already introduced into practice, gave results which were frequently, though not always, in favor of the dusting method. Examples of results obtained by the solution method are shown in Text-fig. 1. Ten cuttings were treated at each of five concentrations, the bases being soaked in the solutions for 22 hours before planting. The curves show the number which rooted in each case. It should be noted that the *Salix pentandra* cuttings (Curves A and B) were dug up after 27 days in sand in the propagating frame, whereas the *Weigela rosea* cuttings (Curve C) were allowed to remain 55 days. The curves indicate a narrower range of tolerance in the solution method than was found in the dusting method.

Responses of Lower Plant Forms

The uniformly positive results obtained with all higher plants tried and the casual observation of similar effects on certain algae, not unlike those found experimentally with algae and just reported by Brannon (1), suggested their applicability to lower forms of plant life. A preliminary series of experiments was carried out on the fermentation of sugar solutions by bakers' yeast. An example of the results is shown in Plate II, Fig. 15, a photograph taken an hour after inoculation, the tubes having stood at a temperature of about 25° C. Gas production was greatly accelerated by 1 p.p.m. naphthylacetic acid (left) and to a lesser extent by 5 p.p.m. (centre) as compared with the control (right). In other experiments 1/10 p.p.m. gave considerable stimulation, and as little as 1/250 p.p.m. had some effect. Higher concentrations than those mentioned have a repressive action.

Similar responses have been obtained with indolylacetic acid, its propionic and butyric homologues, and with the potassium salt of naphthylacetic acid.

Discussion

The hormone treatment of seeds would seem to have important practical applications. The common view that these growth substances repress root growth does not hold when a suitable method of application is used. Experiments in these laboratories, by Dr. R. Newton and Mr. W. R. Jack, have shown that as little as 1/200 p.p.m. naphthylacetic in solution culture depresses the growth of wheat roots in length, though not in weight. Even soaking the seed in hormone solution before planting may have an inhibitory effect on the roots. On the other hand, the dust method seems to be peculiarly adapted to the convenient regulation of hormone supply in such a way as

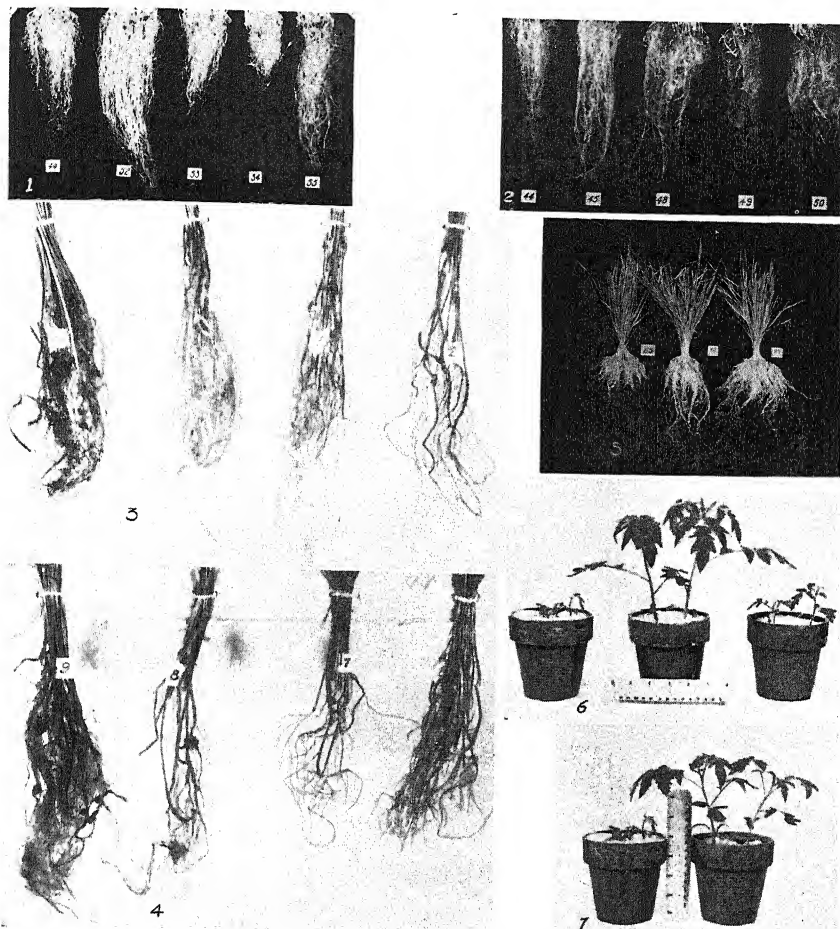
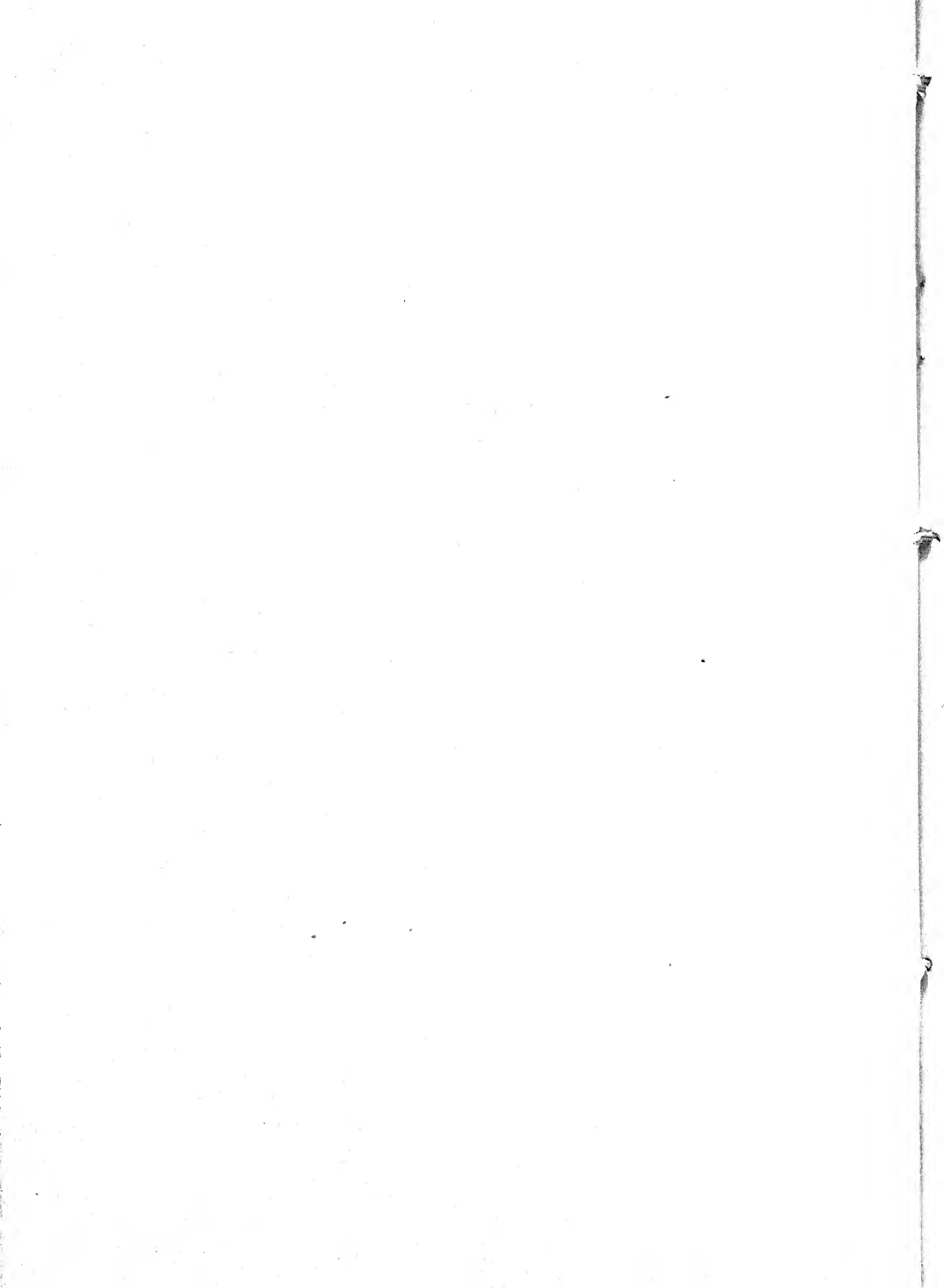


FIG. 1. Barley roots from dust-treated seed, grown in sand 14 days. Left to right: 0, 2.5, 12.5, 62.5 p.p.m. naphthylacetic acid; and 2.5 p.p.m. each naphthyl- and indolyl-acetic acids. FIG. 2. Barley roots from dust-treated seed, grown in sand 14 days. Left to right: 0, 2.5, 25, 50, 125 p.p.m. indolylacetic acid. FIG. 3. Soya bean roots from dust-treated seed, grown in soil six weeks. Left to right: 10 p.p.m. naphthylacetic acid; 20 p.p.m., 5 p.p.m. indolylacetic acid; control. FIG. 4. Soya bean roots from treated seed, grown in soil six weeks. Left to right: 10 p.p.m. indolylacetic acid in talc dust; 100 p.p.m., 10 p.p.m. indolylacetic acid in solution; control. FIG. 5. Wheat seedlings from dust-treated seed, grown in sand 16 days. Left: Control; centre: 5 p.p.m. naphthylacetic acid; right: 5 p.p.m. potassium salt of same. FIG. 6. Tomato plants, 8 days in soil, then 19 days treatment in sand culture. Left: 50 cc. daily nutrient solution only; centre: same, containing 1/100 p.p.m., and right: 1 p.p.m. naphthylacetic acid. FIG. 7. Same control plant (left) as in Fig. 6, and (right) 50 cc. daily of 1/20 p.p.m. indolylacetic acid.



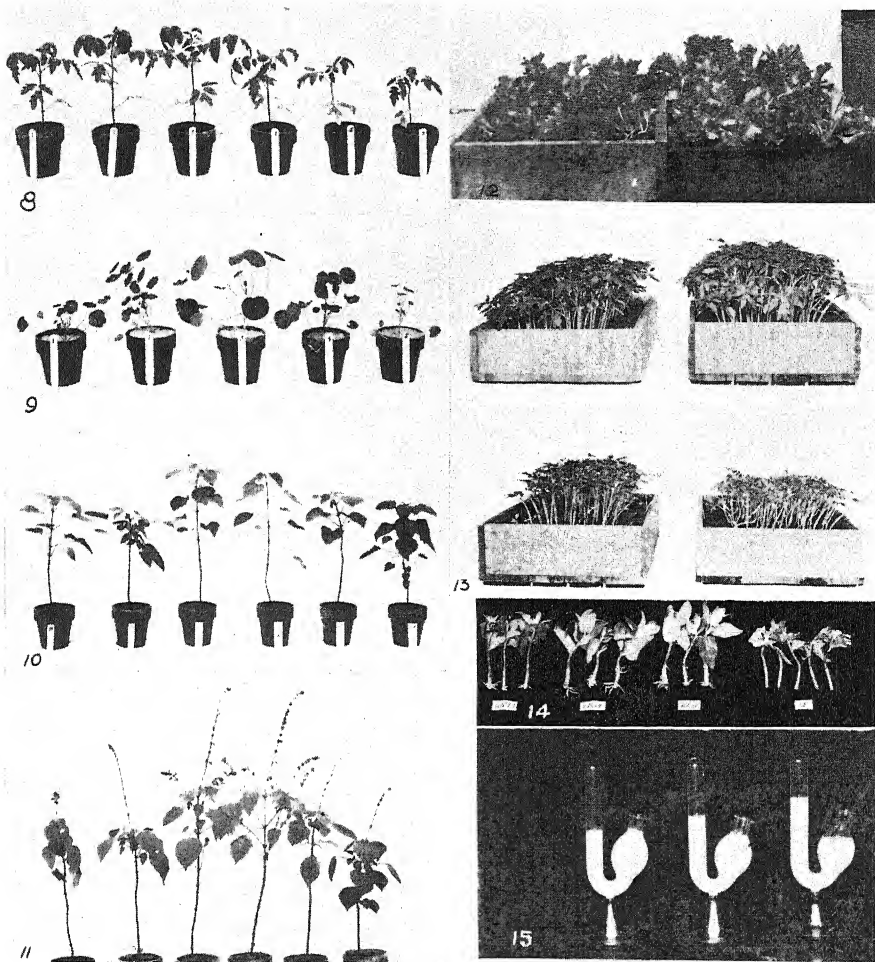


FIG. 8. Tomato plants grown in soil 45 days, treatment last 17 days. Left to right: 0, 1/100, 1/10, 1, 2.5, 10 p.p.m. naphthylacetic acid. FIG. 9. Nasturtiums, 14 days in soil, then 23 days treatment in sand culture. Left to right: 50 cc. daily nutrient solution only; same, containing 1/100, 1/10, 1, 2.5 p.p.m. naphthylacetic acid. FIG. 10. *Salvias* in soil, eight weeks after one treatment. Left to right: 0, 10, 5, 2.5, 1.25, 0.1 mg. naphthylacetic acid per pot. FIG. 11. Same plants as in Fig. 10, taken five weeks later. FIG. 12. Lettuce in soil. Left: control; right: 11 applications at 3-day intervals, of 50 cc. 1/100 p.p.m. naphthylacetic acid. FIG. 13. Tomatoes in soil. Top left: control; right: 1/100 p.p.m. Lower left: 1 p.p.m.; right: 5 p.p.m. naphthylacetic acid, 50 cc. daily for 16 days in all cases. FIG. 14. Dust-treated cuttings of *Deutzia crenata*, 26 days in sand. Left to right: 1000 p.p.m. indolylbutyric, indolylacetic, naphthylacetic acids; untreated control. FIG. 15. Fermentation of 10% sucrose solution by baker's yeast. Left to right: 1 p.p.m., 5 p.p.m., 0, naphthylacetic acid.

to produce positive stimulation of both root and top growth. Whether the increased early growth so clearly demonstrated in the laboratory and greenhouse can be translated to increased yield of crops in the field, can be demonstrated only by field experiments. Institutions co-operating with this laboratory have included tests with dust-treated winter wheat in this fall's planting. It is hoped that various spring crops can be adequately tested next season.

The field application of "hormonized" fertilizers, or the direct application of hormones to greenhouse or garden crops by spraying or otherwise, also holds interesting practical possibilities. With certain young plants, dosages of the order of 100 mg. per acre have been shown to be effective. However, the optimum dosage will vary with a number of factors, such as method of application, composition of the soil, and meteorological conditions, in addition to the kind and age of plant. There is undoubtedly marked adsorption of these active chemicals by soil. Stimulation is effected by an optimum amount of free chemical and that, of course, can be attributed to an equilibrium of some description at the soil-root interface. Our results suggest that for optimum stimulation the available amount of hormone at this interface must be substantially lower than 1/100 of a part per million, almost certainly very much lower than this value.

Greenfield (4) lately reported optimum stimulation of *Matthiola incana* by indolylacetic acid applied to the soil in seven-inch pots at a rate which works out to about 2000 *grams* per acre. This lies in the range of about 1500 gm. per acre found most effective with the *Salvias* shown in Figs. 10 and 11 (Plate II). On the other hand, the effective amounts in the experiments with young lettuce and tomato plants illustrated in Figs. 12 and 13 (Plate II) lie in the radically different range of 50 to 250 *milligrams* per acre. Co-operative field experiments to test the practical utility of these effects are planned for next season.

The dust treatment of cuttings has so far indicated a balance of superiority over the solution method, with respect to effectiveness. Even if we assume it to be no better in its results, its simplicity and convenience still recommend it for practice. Further experiments may prove the one treatment more suitable for certain species or conditions, and the other better adapted to certain other cases.

The marked swelling of herbaceous cuttings when the cut end is immersed in hormone solutions not only gives a clue to the possible mechanism of hormone action in the plant: it also suggests other useful applications. Immersing the base of partly wilted lettuce plants and cut flowers in appropriate concentrations causes these to regain their turgor and freshness in a remarkable way. The life of certain cut flowers may thus be prolonged.

The response of lower plant forms to hormones suggests their possible utility in industrial processes and fermentations. This field is being explored by a number of other workers in these laboratories. It is of incidental interest

that the quick response of yeast has already led to its use by the writer in assaying quickly and conveniently the activity of a mixture of 1- and 2- γ -naphthylbutyric acids, prepared by Dr. R. H. Manske in these laboratories.

Acknowledgments

The indolyl compounds were prepared by Dr. R. H. Manske, the naphthylacetic acid by Dr. A. Cambron, both of these laboratories. Grateful acknowledgment is made to them for large supplies of these chemicals in pure form during the past year.

The writer wishes to express his appreciation of the assistance given by Dr. R. Newton, Director of the Division of Biology and Agriculture.

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OVERWINTERING OF CERTAIN CEREAL PATHOGENS IN ALBERTA¹

By W. R. FOSTER² AND A. W. HENRY³

Abstract

Helminthosporium sativum, *Fusarium culmorum*, *Ophiobolus graminis*, *Leptosphaeria herpotrichoides*, *Wojnowicia graminis*, *Erysiphe graminis*, *Tilletia caries*, and *Tilletia foetens* readily overwinter under natural conditions at Edmonton, Alberta, Canada. The first five of these overwinter at Edmonton in both spore and vegetative stages and are highly resistant to cold. Even in a non-hardened condition several of them survived severe frost. Young germ tubes of *H. sativum* for instance continued growth after being frozen solid overnight. Fresh agar cultures of *H. sativum*, *F. culmorum* and *O. graminis* grew vigorously after exposure to sub-zero temperatures. Agar cultures of *H. sativum* and *F. culmorum* were viable after a 17-day exposure to temperatures ranging from about 0° F. to -50° F.

Conidia of *H. sativum* proved less resistant to freezing and thawing than to continuous freezing. They survived longer than conidia of *F. culmorum* and *F. graminearum*. Mycelia of all foot-rot fungi grown on sterilized barley seeds were viable in one case after three months of continuous freezing, and in another after 40 alternate freezings and thawings. *H. sativum* and *F. culmorum* growing in soil survived 61 alternate freezings and thawings.

H. sativum, *F. culmorum* and *L. herpotrichoides*, retained their viability more readily on the soil surface than when buried at depths of from 2 to 12 in. Well aerated soil seemed to favor the survival of *H. sativum*, although other factors besides aeration probably are involved. Strains of *H. sativum* from high latitudes were not better adapted to low temperatures than strains from lower latitudes.

The bunt fungi, *T. caries* and *T. foetens*, are shown to be capable of overwintering at Edmonton in the form of mycelia in winter wheat. Infection of winter wheat from soil-borne spores may occur in western Canada, but in these experiments soil-borne spores did not survive to infect wheat in the spring.

Erysiphe graminis overwinters in the perithecial stage at Edmonton. In the studies made, ascospores were differentiated in the spring, when favorable conditions prevailed and before the first infections of winter wheat were observed.

Introduction

It is important to know in what form and under what conditions plant pathogenic organisms overwinter. There is little specific information of this sort about pathogens affecting crop plants in western Canada, where winters are as severe as in any other large wheat-growing area of the world. Plant pathogens may overwinter in vegetative or reproductive stages or in both, in or on the soil, the seed, living plants, or plant residues.

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The following pathogens were studied in these investigations:—

A. Foot-rot fungi:—

Fusarium culmorum (W. G. Sm.) Sacc., *Fusarium graminearum* Schwabe, *Helminthosporium sativum* P. K. & B., *Leptosphaeria herpotrichoides* De Not.*, *Ophiobolus graminis* Sacc., *Wojnowicia graminis* (McAlp.) Sacc. & D. Sacc.*

B. Bunt or stinking smut fungi:—

Tilletia caries (D.C.) Tul., *Tilletia foetens* (Berk. & Curt.) Trel.

C. Powdery mildew fungus:—

Erysiphe graminis D.C.

Overwintering of Fungi Causing Foot Rots and Other Diseases

LITERATURE REVIEW

The foot rot and seedling blight of wheat caused by *Fusarium* spp. develops, according to Dickson (6), from two main sources, infected seed and infested soil. Henry's results (12) indicate that in Minnesota the mycelium and possibly some of the spores of *Fusarium* spp. overwinter on debris in the soil or on its surface. Atanasoff (2) states that species of *Fusarium* are remarkably resistant to desiccation and low temperatures and assumes that they overwinter in winter crops as mycelium, conidia and chlamydospores.

Henry (12) found that a rather large proportion of conidia of *Helminthosporium sativum* overwintered on debris in the soil or on its surface at St. Paul, Minnesota. Christensen's results (3), also obtained at St. Paul, Minnesota, indicate that mycelia and spores of *H. sativum* overwinter in the field on old straw, roots, seed, and in the remains of grasses.

Ophiobolus graminis, the fungus that causes the destructive "Take-all," was found by Kirby (15) in New York State, and by Davis (5) in Wisconsin, to overwinter in both ascospore and mycelial stages. Kirby found that winter wheat was damaged most and he considered that the organism lived over the winter on infected plants.

Leptosphaeria herpotrichoides, found in Alberta by Henry and Foster (13), is favored by a mild damp winter in France according to Guyot (10).

OVERWINTERING OF FOOT ROT FUNGI UNDER NATURAL CONDITIONS ON CROP RESIDUES

Overwintered wheat stubble from fields badly diseased with foot rot was collected. Spores of three of the foot-rot fungi, namely, *Leptosphaeria herpotrichoides*, *Ophiobolus graminis* and *Wojnowicia graminis* were present on some of the collections. These were tested for germination in Van Tieghem cells. Pieces of stubble from the various collections were surface sterilized and plated on potato-dextrose agar to determine the viability of any mycelium

* Weakly pathogenic as compared with the others.

present in them. The fungi that developed were checked for identity with monosporous known cultures of the various foot-rot organisms on the same medium.

The mycelium of all organisms mentioned in Table I survived the winter on wheat stubble. A few of the ascospores of *L. herpotrichoides* and pycnosporos of *W. graminis* were viable in the spring but positive results with the ascospores of *O. graminis* were not obtained. However, Davies (4) has since shown that the ascospores of *O. graminis* overwinter at Edmonton. No conidia of *H. sativum* and *F. culmorum* were observed on the stubble examined but, as is shown later, these readily overwinter here.

TABLE I
SURVIVAL OF FOOT-ROT FUNGI UNDER NATURAL
CONDITIONS ON WHEAT STUBBLE DURING
THE WINTER OF 1928-29

Fungus	Isolation of mycelium	Spore germination
<i>Fusarium culmorum</i>	+	—
<i>Helminthosporium sativum</i>	+	—
<i>Leptosphaeria herpotrichoides</i>	+	+
<i>Ophiobolus graminis</i>	+	—
<i>Wojnowicia graminis</i>	+	+

+ Spores and mycelium viable.

— no spores viable.

— No tests made.

COLD RESISTANCE UNDER ARTIFICIAL CONDITIONS

Under natural conditions, pathogenic fungi probably harden off in the same way as higher plants with the gradual onset of cold weather. They also cease growth, and frequently go into dormant or resistant stages. Hence it might be expected that they would survive freezing more readily under such conditions than if kept previous to freezing in a warm atmosphere. In order to test the cold resistance of foot-rot fungi in what would seem to be their most vulnerable states, experiments were made with freshly germinated spores and with fresh agar cultures grown in the laboratory at room temperature.

Effect of Freezing on Young Germ Tubes

In order to determine the resistance of young germ tubes to freezing, spores of *H. sativum* were chosen and germinated in water in Van Tieghem cells. When the germ tubes were from one to three times the length of the spores, the cells were placed outside overnight at freezing temperatures. The minimum temperature reached during exposures was 6° F. The drops of water containing the spores were of course frozen solid. In the morning the cells were returned to the laboratory where the ice soon melted. By the use of several microscopes a number of the germinated spores were then kept under observation to determine the behavior of their germ tubes following freezing. With the aid of a camera lucida a few typical germinated spores were drawn before and after freezing. Some of these are illustrated in Fig. 1.

As Fig. 1 shows, freezing did not kill the germ tubes or injure them severely, though it did tend to induce lateral branches and the formation of new germ

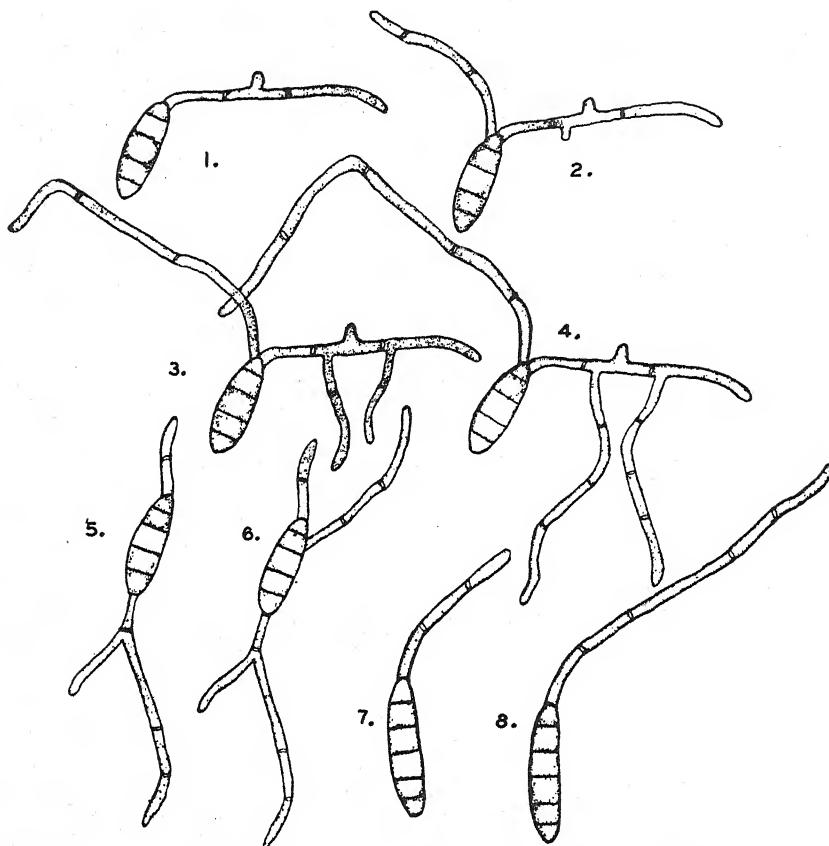


FIG. 1. Effect of freezing fresh germ tubes of *Helminthosporium sativum* in ice over-night. 1-4. Different stages of one germinated spore before and after freezing. No. 1 shows the stage at which freezing took place; 2-4 show stages of growth after thawing. 5-6. No. 5 shows the stage at which another germinated spore was frozen and No. 6 one stage of growth after thawing. 7-8. No. 7 shows the stage at which a third germinated spore was frozen and No. 8 shows the same germinated spore later when the germ-tube had elongated by growth from the tip.

tubes, indicating some damage to the growing tips. However, continuation of new growth from the tip was observed in a few cases. Similar results were obtained with *Fusarium culmorum*.

Effect of Freezing on Growing Cultures on Agar

In order to test the ability of *H. sativum*, *F. culmorum* and *O. graminis* to survive freezing when in a growing condition, potato-dextrose-agar cultures grown in glass test tubes, in the laboratory at room temperature, were exposed outside to freezing temperatures and tested for viability immediately after thawing. If growth was resumed following transfer to fresh slants of potato-dextrose agar, of portions of colonies that had been frozen, tests were considered positive.

Test No. 1. Cultures of *H. sativum* and *F. culmorum*, about a week old, were placed outside on a window ledge overnight. The minimum tempera-

ture reached during the night was 17° F. The cultures were taken into the laboratory at 9 a.m. next morning, allowed to thaw and then tested for viability. Both fungi grew vigorously after this exposure.

Test No. 2. Cultures of *H. sativum*, *F. culmorum* and *O. graminis*, approximately two weeks old, were exposed outside for two hours in a thermograph box about 1½ ft. above the surface of the ground. The thermograph registered -4° F. at the beginning and 0° F. at the end of the exposure. All three fungi grew well after this exposure to below-zero temperatures.

Test No. 3. Cultures of *H. sativum*, *F. culmorum*, and *O. graminis*, 26 days old, were put outside in the same position as the cultures in Test 2, and left there for a period of 3½ days. The temperature was -29° F. at the beginning of the exposure and continued below zero throughout, gradually rising to -1.5° F. at its termination. Tests for viability after the exposure were positive for all three fungi.

Test No. 4. For this test, month-old cultures of *H. sativum* and *F. culmorum* were used. They were placed outside in the same position as the cultures in Tests 2 and 3, at 9.30 a.m. Feb. 4, 1936, and remained there until 3 p.m. on Feb. 21, 1936, when they were returned to the laboratory, allowed to thaw, and tested for viability. The period chosen for this test happened to be one of exceptionally prolonged cold weather (see Table II). For

TABLE II
MAXIMUM AND MINIMUM AIR TEMPERATURES (°F.) RECORDED IN THE OPEN AT THE UNIVERSITY OF ALBERTA DURING FEBRUARY, 1936

	Record A*		Record B*		Record C*	
	max.	min.	max.	min.	max.	min.
Feb. 4	-18	-45	- 4	-40	-18.5	-39
5	-19	-43	-14	-38	-20	-38.5
6	-29	-39	-14	-30	-29	-36
7	-24	-47	-22	-42	-30	-41.8
8	-15	-43	-14	-30	-17	-31
9	-15	-24	- 2	-18	-15.5	-19
10	-13	-21	0	-20	-14	-23
11	-15	-33	- 4	-29	-13.5	-19
12	-14	-41	- 2	-39	- 9.5	-36
13	-14	-43	- 2	-38	-12	-42
14	-22	-41	- 8	-36	-21	-38.8
15	-23	-50	-13	-46	-14	-45.5
16	-17	-56	-10	-50† (?)	-21	-51
17	-12	-45	- 4	-26	- 9.5	-26.7
18	-10	-38	+ 2	-34	- 5.5	-36
19	- 4	-34	+ 7	-20	- 3	-23.5
20	- 2	-24	0	-20	- 1.5	-23
21	+ 1	-20	+17	-11	+ 3	-13

*Record A. Max. and min. thermometer, Experimental Plots, Field Crops Dept., University of Alberta, Edmonton. (Courtesy Mr. J. W. Hopkins).

Record B. J. P. Friez thermograph, University of Alberta Campus, near West Lab. †estimated—pen below chart.

Record C. Max. and min. thermometer, University of Alberta Campus, near North Lab., Dept. of Civil Engineering. (Courtesy Prof. H. Webb).

instance, a minimum temperature of at least -50°F. was recorded at the University by two maximum and minimum thermometers and a thermograph, on the night of Feb. 16, and the temperature was consistently below zero for most of the period. *H. sativum* and *F. culmorum*, however, grew vigorously following the exposure.

The results of these tests clearly demonstrate that non-hardened cultures of *H. sativum*, *F. culmorum* and *O. graminis* are highly resistant to low temperatures. Under natural conditions these fungi are not likely to be exposed to such severe conditions. In most situations where they occur, for instance, on stubble and other crop residues, they are covered during much of the winter by a protective blanket of snow. Moreover, under natural conditions, they would usually have an opportunity of becoming more resistant to cold owing to a hardening process in the fall. Hence if these organisms can survive the above conditions, it might be expected that they would be successful, as has been demonstrated, in overwintering under natural conditions.

Effect of Continuous and Alternate Freezing and Thawing

During an Alberta winter, organisms under outside conditions may be exposed to wide variations in temperature both above and below the freezing point. If there is no protective cover, the fluctuations will be especially wide, particularly in the spring or during occasional warm periods or "chinooks." On the other hand, if the organisms are under snow, soil, or other cover, they will sometimes be exposed to less extreme and relatively constant temperatures for long periods.

In order to determine the relative effects of continuous freezing and alternate freezing and thawing on foot-rot fungi, cultures on sterilized barley kernels were frozen in ice outside, and in a refrigerator at -5°C. Each morning half of the outside cultures were brought into the laboratory and allowed to return to room temperature. As soon as the ice melted they were put out to freeze again. The other cultures were kept frozen continually and tested for viability at intervals. Data were taken on both spores and mycelia.

In one experiment a culture of *H. sativum* was tested over a period of eight weeks. A portion of it was kept frozen solid during this period while the remainder was frozen and thawed forty times. The results of viability tests of representative samples of this material are given in Table III.

It will be noted from the above table that intermittent freezing killed the spores of *H. sativum* more rapidly than did continuous freezing. The conidia survived twice as long when kept frozen as they did when frozen and thawed almost daily. In this connection, Christensen (3) found that conidia of *H. sativum* survived freezing and thawing in water for a considerable time. The mycelium in the above experiment evidently survived after the spores were killed, as it was viable in all tests as indicated by positive results throughout the vegetative growth. This may have been due to its presence within the barley kernels where it may have been less subject to injury than on the surface.

TABLE III

EFFECT OF CONTINUOUS AND INTERMITTENT FREEZING ON THE VIABILITY OF *H. sativum*
GROWN ON STERILIZED BARLEY SEEDS

Periods of testing	Number of times frozen and thawed	Germination of <i>H. sativum</i>			
		Continuous freezing		Intermittent freezing	
		Germination of conidia	Vegetative growth	Germination of conidia	Vegetative growth
1 day	1	High	+	High	+
3 days	3	High	+	High	+
1 week	6	High	+	High	+
2 weeks	12	High	+	Low	+
3 weeks	18	High	+	Low	+
4 weeks	24	Medium	+	0	+
6 weeks	36	Low	+	0	+
8 weeks	40	0	+	0	+

In another experiment the effect of continuous freezing of several fungi over a three month period was studied. The results of this experiment are given in Table IV.

TABLE IV

EFFECT OF CONTINUOUS FREEZING ON SEVERAL FOOT-ROT FUNGI GROWN ON
STERILIZED BARLEY SEEDS

Organism	Source	1 month		2 months		3 months	
		Germ.* conidia	Growth myc.	Germ. conidia	Growth myc.	Germ. conidia	Growth myc.
<i>F. culmorum</i>	Edmonton, Alta.	Med.	+	0	+	0	+
<i>F. graminearum</i>	St. Paul, Minn.	Med.	+	0	+	0	+
<i>F. graminearum</i>	Baton Rouge, La.	Med.	+	0	+	0	+
<i>H. sativum</i>	Ft. Vermilion, Alta.	High	+	Low -	+	0	+
<i>H. sativum</i>	Edmonton, Alta.	High	+	Low -	+	0	+
<i>H. sativum</i>	St. Paul, Minn.	High	+	Low -	+	0	+
<i>H. sativum</i>	Baton Rouge, La.	High	+	Low -	+	0	+
<i>L. herpotrichoides</i>	Camrose, Alta.	-	+	-	+	-	+
<i>O. graminis</i>	Camrose, Alta.	-	+	-	+	-	+
<i>W. graminis</i>	Edmonton, Alta.	-	+	-	+	-	+

*Low = 1-10%, Medium = 11-35% = 35-100%.

All the fungi included in the above experiment, namely, *Fusarium culmorum*, *Fusarium graminearum*, *Helminthosporium sativum* (four strains), *Leptosphaeria herpotrichoides*, *Ophiobolus graminis* and *Wojnowicia graminis*, survived the entire three months frozen solid continuously in ice. The spores of all four strains of *H. sativum* survived this treatment for two months, but those of the two species of *Fusarium* were dead in two months and those of *H. sativum* in three months. No spores of *L. herpotrichoides*, *O. graminis* or *W. graminis* were available for testing. Although not indicated in Table IV, all of the fungi mentioned in it also survived alternate freezing and thawing forty times during a two month period.

In another experiment *H. sativum* and *F. culmorum* were grown in sterilized soil instead of barley grains and tested in this medium for resistance to alternate freezing and thawing. In this experiment the cultures were started at room temperature in test tubes containing equal portions of the autoclaved soils, which had previously been made up to their water-holding capacity with distilled water. Three soils representative of the three main soil types of the province were used, namely, black, brown, and gray, with average organic matter contents of 10, 6 and 10. The fungi were allowed to develop in these soils for 17 days at room temperature, after which the test tubes were placed in a refrigerator at 8° C. for 11 days. Viability tests were then made, all of which were positive. Freezing and thawing was then begun. The usual procedure was to place the tubes of soil on the trays between the coils of a refrigerator at a temperature of about -5° C. and to leave them overnight. They were removed about 9 a.m. next day and allowed to remain at room temperature until 5 p.m., when they were again placed in the refrigerator and frozen. They were not melted every day, but on the average on each of about 15 days a month over a period of about four months. In all, the cultures were frozen and thawed 61 times, after which viability tests were made. These were positive for both fungi in each of the soil types. The treatments were not continued, so it is not possible to say how long the fungi will survive such exposures. It is clear, however that they are not readily killed in sterilized soil by alternate freezing and thawing.

LOCATION IN THE SOIL IN RELATION TO WINTER SURVIVAL

Christensen (3) has reported that aeration is an important factor in prolonging the life of conidia of *H. sativum*. This might be one of the factors affecting the survival of organisms deposited in the soil. Aeration would ordinarily decrease with depth and with increasing firmness of the soil. Hence winter survival at different depths in the soil or in soils of varying degrees of firmness might vary because of differences in air supply.

In order to ascertain whether position in the soil and firmness of the soil have any effect on the overwintering of foot-rot fungi, barley seed cultures were placed outside on the surface of Edmonton black soil and also at depths of 2, 4, 8 and 12 in., on November 1, 1928, *Helminthosporium sativum*, *Fusarium culmorum* and *Leptosphaeria herpotrichoides* were used in this experiment. Viability tests were made of the overwintered cultures May 1, 1929. The results of these tests are given in Table V.

The data in Table V indicate that all three fungi survived better on the surface than below ground. The conidia of *H. sativum* kept on the surface also germinated better than those from below ground, though this was not true of *F. culmorum* spores. *H. sativum*, overwintered in unpacked soil, also showed a higher degree of viability than that overwintered in packed soil; but no difference was shown by either *F. culmorum* or *L. herpotrichoides*. In another experiment in which barley seed cultures of *H. sativum* and *F. culmorum* were overwintered in different types of soil, those kept in sand, the

TABLE V

EFFECT OF EXPOSURE OUTSIDE DURING THE WINTER OF 1928-29 OF CULTURES OF FOOT-ROT FUNGI AT DIFFERENT DEPTHS, AND IN PACKED AND UNPACKED SOIL

Treatment	Germination of conidia*		Growth on agar		
	<i>H. sativum</i>	<i>F. culmorum</i>	<i>F. culmorum</i>	<i>H. sativum</i>	<i>L. herpotrichoides</i>
<i>Depths of inoculation</i>					
Surface	High	Low	Excellent	Excellent	Good
2 in. below	Low -	Medium -	Fair	Poor	Fair
4 in. below	Low -	Low	Fair	Poor	Fair
8 in. below	Low -	Low	Fair	Poor	Fair
12 in. below	Low +	Low	Good	Fair	Fair
<i>Firmness</i>					
Packed 4 in.	Low	Low	Fair	Poor	Fair
Unpacked 4 in.	Medium	Low	Fair	Fair	Fair

* Low = 1-10%; Medium = 11-35%; High = 36-100%.

most porous soil, showed a higher percentage germination of their conidia than any of the others. It would appear therefore that good aeration may be a factor favorable to successful overwintering. The above experiments, however, provide no conclusive proof of this. It is possible, for example, that differences in the amount of moisture, and in the activity of soil micro-organisms at different levels, might affect the survival of the pathogenic fungi under consideration.

ECOLOGICAL ADAPTATION

The ability of an organism to overwinter in northern regions might conceivably be due to the occurrence of specially cold-resistant strains in those areas. In order to test this hypothesis, strains of *H. sativum* were obtained from a number of points from Fort Vermilion, Alberta, in the north, to Baton Rouge, Louisiana, in the south. These cultures were grown on potato-dextrose agar and exposed to temperatures ranging from 5° to 35° C. and their reactions measured by taking the average diameters of the colonies.

TABLE VI

REACTION OF DIFFERENT STRAINS OF *Helminthosporium sativum* TO DIFFERENT TEMPERATURES

Source of strains of <i>H. sativum</i>	Diameter of colonies in millimeters at various temperatures						
	5° C.	10° C.	15° C.	20° C.	25° C.	30° C.	35° C.
Fort Vermilion, Alberta	11	15	47	62	88	62	25
Berwyn, Alberta	11	18	32	41	48	76	14
Edmonton, Alberta	11	18	23	49	85	86	25
Ohaton, Alberta	12	15	54	71	88	86	26
Calgary, Alberta	16	23	46	69	88	77	18
Brooks, Alberta	17	25	32	46	72	65	15
Claresholm, Alberta	14	25	45	61	87	60	15
St. Paul, Minnesota	14	18	37	64	75	76	16
Baton Rouge, Louisiana	8	13	30	46	52	58	0

The data presented in Table VI do not indicate that northern strains grow better at lower temperatures than do those from points farther south. The responses of the different strains to temperatures seem to be about the same. The Berwyn and Baton Rouge strains appear to have a slightly higher optimum than the others. Four of the above strains from latitudes ranging from Fort Vermilion, Alberta, to Baton Rouge, Louisiana, reacted similarly to continuous freezing and to alternate freezing and thawing. No definite ecological adaptation is therefore demonstrated in these experiments.

Overwintering of Bunt Fungi

LITERATURE REVIEW

The bunt fungi are known to overwinter commonly in western Canada in the form of chlamydospores adhering to the surface of the seed. There has, however, been a lack of experimental evidence, to show whether the bunt fungi can survive the winter as mycelia in winter wheat in western Canada. Woolman and Humphrey (16) have demonstrated that soil-borne spores may cause a smutty crop of wheat in the Palouse region of Idaho, Washington and Oregon, even though the seed has been carefully treated. However, when spring wheat is sown on similarly infested soil the crop remains practically bunt free, indicating that the spores do not survive the winter in that region. Appel and Riehm (1) and others have also reported the failure of free spores to overwinter. In western Canada, Güssow and Conners (9) point out that "As a rule threshing is done so late in the fall the soil is too cold to permit the germination of any spores which have been set free by this operation." They call attention, however, to the effectiveness of seed treatments in controlling bunt of spring wheat and consider that this indicates that infection from spores which have overwintered in the soil does not commonly occur. Hanna and Popp (11) found that bunt spores (*Tilletia caries*) in infected heads of Mindum wheat placed on the surface of the ground overwintered successfully at Winnipeg, Morden, Brandon, Indian Head, Saskatoon and Edmonton, in western Canada.

EXPERIMENTAL RESULTS

On September 6, 1928, field plots were laid out at Edmonton, and bunt spores were distributed both in rows and broadcast. Treated and non-treated Kharkov winter wheat were seeded in half of the plots. On April 26, 1929, the remaining half was sown to Marquis wheat. Temperature and moisture conditions were favorable for infection both in the fall and spring. When the grain was nearly mature the percentage of bunted heads was recorded and specimens collected to determine the species that overwintered.

Table VII shows that winter wheat sown in the fall in soil infested with bunt spores became affected with bunt, while spring wheat did not. Soil-borne spores of both *Tilletia caries* and *Tilletia foetens* caused infection of winter wheat. The bunt spores evidently did not survive the winter in the soil, but the mycelium in winter wheat plants readily overwintered. It is

TABLE VII
EFFECT OF SOIL INFESTATION WITH CHLAMYDOSPORES ON THE DEVELOPMENT
OF BUNT IN WINTER AND SPRING WHEAT

Seed treatment	Soil treatment	Winter wheat Heads infected, %	Spring wheat Heads infected, %
Check	No inoculum	0.0	0.0
Untreated	Inoculum added to rows	14.0	0.0
Copper carbonate	Inoculum added to rows	9.7	0.0
Formaldehyde	Inoculum added to rows	10.0	0.0
Hot water	Inoculum added to rows	10.0	0.0
Check	No inoculum	0.0	0.0
Untreated	Inoculum broadcasted	13.5	0.0
Copper carbonate	Inoculum broadcasted	8.5	0.0
Formaldehyde	Inoculum broadcasted	10.7	0.0
Hot water	Inoculum broadcasted	10.0	0.0

also of interest to note that the infection of winter wheat was practically as great in the plots where the spores were broadcasted as in those in which the spores were deposited in the seed rows. There seems, therefore, to be a good possibility of winter wheat becoming infected with bunt in Alberta from soil-borne spores. Seed treatment, as shown by Table VII, will not prevent this type of infection. The greater prevalence of bunt in winter wheat as compared with spring wheat in Canada, may be accounted for by the above results. According to estimates of the Board of Grain Commissioners of Canada, winter wheat in Canada had proportionally about 70, 50 and 18 times as much bunt as spring wheat in 1926, 1927 and 1928. Furthermore, contrary to the suggestions of Güssow and Connors (9), fall conditions at threshing time and after are often favorable for germination of bunt spores. The mean monthly air temperature for September at Edmonton for the last 47 years was 50° F. According to Hungerford (14), Gibs (7), Güssow and Connors (9) and others, maximum infection takes place between 46° and 50° F. Further, the average soil temperature two inches below the surface for the last three weeks in September, 1928, was even somewhat higher than the optimum soil temperature for infection.

It appears that temperature conditions in the fall at Edmonton are generally favorable for infection of winter wheat from soil-borne bunt spores.

Overwintering of the Powdery Mildew Fungus of Wheat

Erysiphe graminis D.C., the fungus causing powdery mildew of grasses, occurs commonly in the moister parts of Alberta on wheat and rye as well as on many wild and cultivated grasses. Winter wheat and winter rye are usually the cereals most heavily attacked, but spring wheat is frequently and sometimes quite severely affected. Powdery mildew has not been observed at Edmonton in the field on oats and barley, though the disease developed on oats in the greenhouse on one occasion.

Both conidial and perithecial stages of *Erysiphe graminis* develop in abundance at Edmonton on affected plants. In order to determine whether the fungus overwinters on wheat in either or both of these stages, the behavior of

each was followed during the fall, winter, spring, and early summer of 1928-29.

TABLE VIII
VIABILITY OF CONIDIA OF *Erysiphe graminis* FROM WINTER WHEAT COLLECTED IN THE FALL OF 1928

Date of collection	Germination of conidia, %
Oct. 1	11
Nov. 1	0
Dec. 1	0

Collections of conidia from winter wheat were made at monthly intervals during the fall. These were brought to the laboratory and tested for germination on the day of collection. The results of the tests are given in Table VIII.

It will be seen that viable conidia were present on winter wheat on Oct. 1, but after that no germination occurred. The conidia not only failed to germinate in the

late fall but were difficult to find, which suggests that they are not concerned in the overwintering of the fungus here.

Perithecia were also collected from winter wheat during the fall of 1928 and at various times thereafter up to June 15, 1929. Diseased leaves were brought to the laboratory at each date of collection and perithecia from them were crushed and examined under the microscope for the presence of asci containing mature germinable ascospores. As is indicated in Table IX, no perithecia having asci with differentiated ascospores were found until May 15. By the second week in June numerous mature ascospores were found in the perithecia examined. The first infections of winter wheat observed in 1929 were found on June 15. Thus the maturing of the ascospores corresponded very well with the appearance of the initial infections. This, coupled with the failure of the conidia to survive after October, indicates that the fungus overwinters here in the perithecial stage.

TABLE IX
DEVELOPMENT OF ASCOSPORES OF *Erysiphe graminis* IN THE FIELD AT EDMONTON IN 1928-1929

Date	Ascospores formed
Oct. 1	—
Nov. 1	—
Jan. 3	—
Feb. 4	—
March 1	—
April 1	—
May 1	—
May 15	+
June 1	+
June 8	++
June 15	++

— No ascospores differentiated;
+ ascospores beginning to differentiate;
++ ascospores differentiated.

TABLE X
AVERAGE MAXIMUM AND MINIMUM DAILY TEMPERATURES, ° F., AT EDMONTON, BY WEEKLY PERIODS FROM MAY 1 TO JUNE 15, 1929

Week ending	Average temperatures, ° F.	
	Maximum	Minimum
May 7	55.6	29.0
14	57.7	35.9
21	66.9	37.6
28	65.6	42.0
June 4	70.9	46.9
11	78.3	47.9
18	71.9	47.3

Graf-Marín (8) has recently investigated methods of breaking the dormancy of perithecia of *Erysiphe graminis*. Best results were obtained by chilling the perithecia, immersed in water, for 12 hr. at 9° C. (48.2° F.) and then transferring them to a constant temperature of 21° C. (69.8° F.). Ascospores were then formed in 22 hr.

An examination of daily maximum and minimum temperature records at the University of Alberta, Edmonton, for May and the first two weeks of June, 1929, reveals a gradual rise during May to temperatures at the end of the month and during the first two weeks of June, closely approaching those reported as optimum for ascospore differentiation by Graf-Marín (Table X). As already noted and as may be seen in Table IX, most ascospore differentiation was actually observed on June 8 and June 15.

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THE ORIGIN OF RUSSETING IN THE GOLDEN RUSSET APPLE¹

BY HUGH P. BELL²

Abstract

About the time of full bloom, many epidermal cells divide by a tangential wall. Later in June all the epidermal cells become vacuolated and some divide again by tangential walls forming a layer varying from two to four cells thick. Early in July a cambium is initiated in the innermost cells of epidermal origin. This cambium is very active and immediately gives off cells which differentiate into cork. Non-russeted portions may have either a very thick convoluted cuticle or a double layer of cuticle. The development of the periderm and the histology of the mature protective layers are illustrated by fifteen figures.

Introduction

During the spring of 1936, the Pathologist-in-Charge at the Laboratory of Plant Pathology, Kentville, Nova Scotia, was studying the question of russeting on certain varieties of apples. His immediate problem was the pathological aspects of this phenomenon, but before a satisfactory diagnosis could be made of the pathological condition, it was considered desirable to have more information regarding russeting as it occurs normally on certain apples. The study outlined below was undertaken to obtain this information.

Historical

The development of normal russeting is described briefly by Zschokke (7). He also has a few figures illustrating its origin and development. In agreement with the findings given below, he states that the cork cambium cells arise from the inner half of epidermal cells, but his description of the histology and development is very brief and his figures are almost idealistic in their regularity of tissues and perfection of cells. Thus his article was of little assistance in identifying the tissues in the material collected at Kentville. Recent reports on russeting that arose as the result of injury describe the corky or periderm layer as originating in sub-epidermal layers. For instance MacDaniels and Heinicke (4, p. 905) state ". . . . the wound has been corked over by the activity of a periderm layer formed in living cells beneath the injured tissue." Also Clements (3) reports that in the rare cases where real cork occurs in the lenticels of the apple, it develops from the inner cells beneath the ruptured epidermis. In all these cases the mature periderm is apparently similar in structure, regardless of whether it arises normally, or as the result of injury, or in the development of a lenticel. Hence, as recent investigators agreed that the periderm of injuries and lenticels arose from inner tissues, and as the figures of Zschokke did not agree in detail with the material collected at Kentville, it was considered necessary to make a careful study of the subject. This was done not only to see whether Zschokke was

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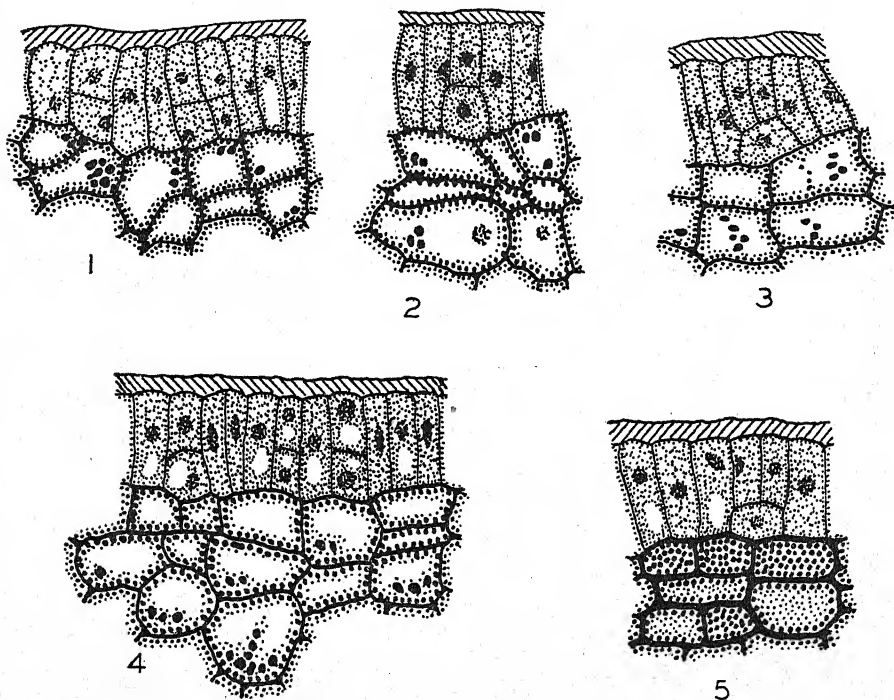
correct when he stated that normal russeting in the apple originated in the epidermis, but also to get more detailed information regarding the histology of russetting.

Material and Methods

The variety of apple used was the Golden Russet. Collections were made biweekly from normal trees in the orchard of the Experimental Farm at Kentville, Nova Scotia. The material was killed in chrom-acetic, imbedded in paraffin as described by Bell and Facey (2) and stained in safranin and fast green. The section thickness that proved most satisfactory was 6μ . Sections thicker than this were useless for accurate interpretation of tissue development. In 1936 the trees from which the material was collected were in full bloom during the last week in May.

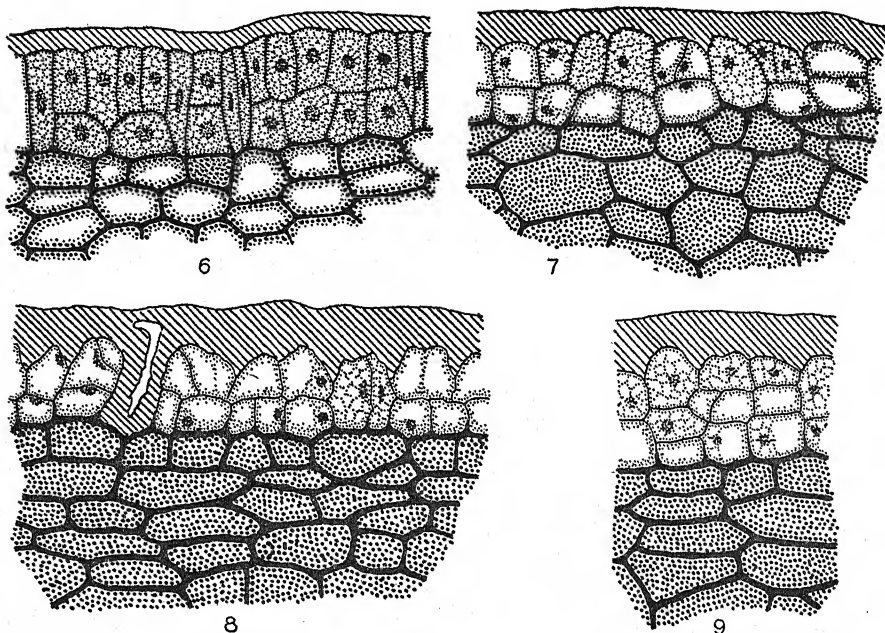
Development and Histology

Before and during full bloom, the epidermal structure of the Golden Russet is very similar to that found during the same period in the McIntosh Red. This has been described and illustrated by Bell (1). That is, at full bloom



FIGS. 1-5. Radial sections through outer layer; cuticle singly cross-hatched. FIG. 1. June 1. Three of the epidermal cells have divided by tangential walls. $\times 550$. FIG. 2. June 4. The inner half of a divided epidermal cell has broadened slightly tangentially. $\times 550$. FIG. 3. June 4. The outer half has divided by a radial wall and the inner half has broadened considerably tangentially. $\times 550$. FIG. 4. June 4. Three cells have divided transversely, and the inner half of the left hand divided cell has broadened tangentially. $\times 550$. FIG. 5. June 8. Similar to Fig. 3, but the cells have assumed a different shape. $\times 550$.

the epidermis consists of closely packed columnar cells with a radial measurement a little more than twice the tangential. The variation from the normal which gives the Golden Russet its distinctive character starts during and shortly after full bloom. It consists of tangential divisions in the epidermal cells. At first the tangential walls occur in a very few cells. In nearly all cases they are slightly nearer the inner than the outer end of the cell (Figs. 1, 4). As this transverse division becomes more common throughout the epidermis, the inner half broadens in a tangential direction (Figs. 2, 4). At the same time, the outer half frequently divides by a radial wall (Figs. 3, 5). This radial division of the outer cell obscures the origin of the inner half, and as this inner half has broadened tangentially and flattened radially, it has become quite similar in appearance to the cells of the hypodermis, and unless the earlier stages have been observed very carefully, it is very easy at this stage to make a mistake and regard the inner cell of the epidermis as sub-epidermal in origin. While this development in the epidermis is taking place, the cuticle is thickening rapidly. During the early part of June, cell division and cell differentiation continue, until by the middle of the month, nearly the whole epidermis is transformed into a layer two cells thick (Fig. 7).



FIGS. 6-9. Radial sections through outer layer; cuticle singly cross-hatched. FIG. 6. June 11. Transverse division and tangential broadening, especially of the inner cells, has extended throughout most of the epidermis. $\times 400$. FIG. 7. June 18. Most of the epidermis has been transformed into at least two layers of vacuolated cells. $\times 400$. FIG. 8. July 6. A conspicuous hair base, the inside end of which indicates quite clearly the inner boundary of the epidermal layer. $\times 400$. FIG. 9. July 6. A typical example of those regions in which there has been more than one tangential division in the cells of epidermal origin, resulting in a layer more than two cells thick. $\times 400$.

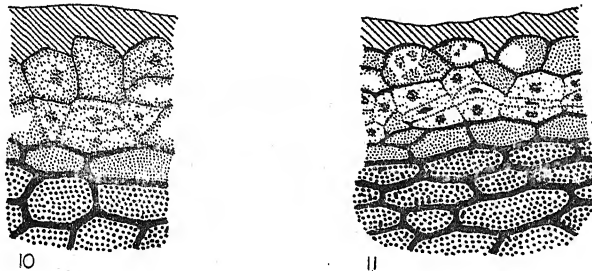
Concurrent with or immediately following the development outlined above, a number of other changes occur. For instance, all the epidermal cells enlarge greatly in a tangential direction and become vacuolated. The outer cells become partially separated by V-shaped invasions of the cuticle. Either the outer or inner cells or both may divide again by a tangential wall. Thus by the first week in July, the epidermis is a layer of broad vacuolated cells. It usually varies in thickness from two to four cells and is covered by a thick and invading cuticle (Fig. 9). But it must be understood that there are still some regions in which no division of the epidermal cell has taken place and there, of course, the epidermis is still one cell thick, but these single cells are usually very large. (See fourth and fifth epidermal cells from the right, Fig. 8.)

In those places where a number of divisions have occurred and the epidermis is three or four cells thick, it has become increasingly difficult to determine with certainty where cells of epidermal origin end, and those of hypodermal origin start. The most trustworthy landmark is the inner end of the hair base. This marks the original inside boundary of the epidermis, for as the epidermis thickens and becomes multicellular, the hair base lengthens and is made conspicuous in transverse section by a heavy deposition of cuticle around the base and sides and finally over the top of this columnar cavity (Fig. 8). By using this hair base as a landmark, it is comparatively easy to determine the boundary between cells of epidermal and cells of sub-epidermal origin.

During the first two weeks in July the whole structure of the epidermal or outer protective layer differentiates very rapidly; so rapidly that it is necessary to make many collections close together to interpret the changes correctly. The exact sequence of change is obscured because in this one group of cells, growth is taking place simultaneously in two directions and by two very different methods. So different are these two methods that they appear to conflict with each other, and yet they are progressing concurrently in the same group of cells. First the ovary is enlarging very rapidly and the outer protective layer must accommodate itself to this enlargement by growth in a tangential direction. To accomplish this the cells stretch tangentially, flatten radially, occasionally divide by a radial wall, and are all displaced and more or less distorted. Many, especially the outer ones, become crushed or even ruptured. This phase of the differentiation is accomplished chiefly by stretching and gliding growth. While this tangential extension, with the consequent distortion and disruption of cells, is progressing, a regular cork cambium layer is being differentiated and cork cells are being cut off and pushed out radially. This phase of the growth is accomplished by rejuvenation of vacuolated cells into meristematic cells, followed immediately by rapid growth in a radial direction; the latter, of course, being the result of cell division in which the new dividing walls are laid down tangentially. Owing to the great differences in these various forms of growth, occurring at the same time in this one layer of cells, portions may become so distorted that it

is often impossible to interpret or explain the cell arrangement. The rapidity with which these changes take place is apparent from the fact that in all the collections before July 6 and in most of the collections on that date (Fig. 9), no trace of cambium or periderm is to be found, but less than a week later, a large part of the surface of the fruit is found to include an active cork cambium and a well formed layer of cork (Fig. 12).

The first trace of cambium is found in a few cells on and after July 6. It appears as a thin radial wall in the inner cell of the epidermal layer (Fig. 10). The cambium cell is initiated always in a cell of epidermal origin and never



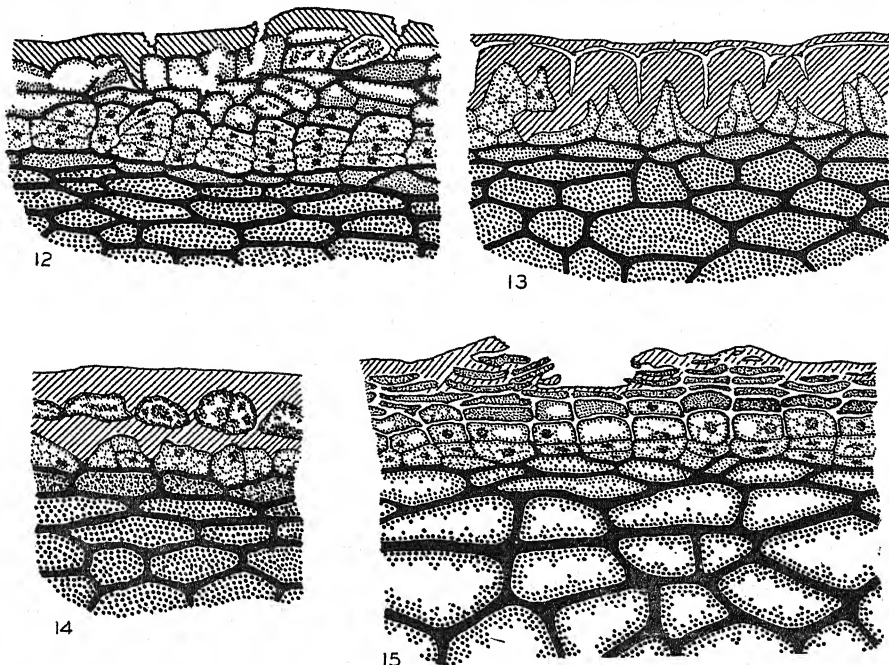
FIGS. 10-11. Radial sections through outer layer; cuticle singly cross-hatched. FIG. 10. July 6. Initiation of the cambium layer. Note that the division is in the innermost cell of epidermal origin, the tangential wall is very delicate or thin and the two resulting cells are typically meristematic in structure. $\times 320$. FIG. 11. July 9. A small patch of recently formed and active cambium. The contents of the outer epidermal cells have started to disintegrate. $\times 320$.

in a cell of sub-epidermal origin. This division of the inner epidermal cell by a radial wall very soon becomes quite common, so that, within a very few days, patches of such cells are found frequently throughout the layer (Fig. 11). These cells immediately start to divide rapidly by tangential walls. The cells given off towards the outside mature and form typical radial rows of cork cells. During this process the outer cells of the original epidermal layer die, their contents disintegrate and their walls rupture. Also the cuticle, by rupturing in many places, forms the first indication of the surface cracks so typical of a russet apple (Fig. 12).

From this stage on there is nothing in the development of the russet layer which is in any way different from the development of any typical periderm or cork. The cuticle remains on the outside, and immediately beneath it are the crushed epidermal cells and the crushed outer cork cells. The ruptures in the cuticle and crushed outer cells enlarge as the fruit enlarges, and form the network of minute cracks so characteristic of the ripe russet apple. These cracks are quite conspicuous in a cross section of the mature periderm (Fig. 15). During July and August, while the fruit is attaining its maximum size, the periderm becomes the dominant outer protective layer. By this time the cork cambium cells form an even tangential row which conforms very little to the unevenness and cracks on the outer surface. As the mature russet layer appears to be typical periderm, the structure of which is so well

known, and adequately described in so many textbooks, there is no need to give a detailed description here.

In the Golden Russet apple there are, of course, portions of the surface which do not become russeted. The structure of the protective layers in these regions is worthy of at least a brief description. In general, the epidermis of the non-russeted portions is either one or two cells thick. Where it is single celled, the cuticle is very thick and it is usually folded or convoluted



FIGS. 12-15. Radial sections through outer layer; cuticle singly cross-hatched. FIG. 12. July 9. A stage slightly more advanced than shown in Fig. 11. The cambium has extended further and in places it has already given off cells which will later become cork. The outer epidermal cells are crushed and ruptured, and the cuticle has cracked in various places. $\times 370$. FIG. 13. Aug. 6. A convoluted cuticle from a non-russeted portion. The epidermal cells have apparently not divided and hence the layer in such a region is chiefly one cell thick. $\times 370$. FIG. 14. Aug. 13. A typical two layered cuticle from a non-russeted portion. $\times 370$. FIG. 15. Sept. 24. Mature periderm or russet, including a cross-section of a typical "crack". $\times 370$.

(Fig. 13). Tetley (5, p. 166, and Fig. 9) reports a cuticle condition like this for the "Lord Suffield" variety. Where the epidermis is two cells thick, the cuticle is often in two distinct layers, with the outer halves of the epidermal cells forming a row of cells between the two layers of cuticle (Fig. 14). Zschokke (7) describes this condition and explains it by saying that the inner half of the epidermal cell assumes the usual function of the epidermis and continues to form cuticle, with the inevitable result that the outer half of the epidermal cell is pushed out. Tetley (6, p. 284, and Fig. 4, c) describes a somewhat similar cuticle structure for the "Bramley's Seedling" variety, and refers to the inner layer of cuticle as "Fatty deposits between the lower

tangential wall of the epidermal cells and the sub-epidermal cells” Whatever the correct description or explanation may be, a cuticle that has the appearance of being in two layers is quite typical of non-russeted portions of the Golden Russet apple.

When examining the protective layers of such an apple as the Golden Russet, it is well to bear in mind that the different structures described above are not always distinct and sharply differentiated from each other. The various types merge one with the other, forming a tissue which is often quite complex.

Conclusion

The net result of this study is that the finding of Zschokke is confirmed. That is, in the apple, normal russetting originates in the epidermis. However there are those, who, like the author, find it necessary to identify these structures, and to them the more detailed descriptions and figures given in the present article should be of considerable assistance.

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THE QUALITY AND GRADING OF FROSTED WHEAT

ANNUAL SURVEYS OF THE 1930 TO 1935 WESTERN CANADIAN CROPS¹

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Abstract

Fifteen officially graded samples of frosted wheat were collected from each of grades No. 3 Northern to No. 6, for 1930, 1931, 1932 and 1933, and 20 samples from each of these grades in 1934 and 1935. Physical classification of the kernels in each sample showed that the present system of grading is efficiently applied. While this system usually gives grades of frosted wheat, the averages of which fall in the right order with respect to combined milling and baking quality, it fails to give close indication of the baking quality, particularly of individual samples.

A statistical examination of the relation between quality characteristics and grading factors showed that milling quality is closely related to the percentages of immature and heavily frosted kernels and also to the weight per measured bushel. Baking quality is not closely related to any of the grading factors now in use. Protein content is the best single index of baking quality, and the relation is improved if the percentages of immature and heavily frosted kernels are taken into account. The quality decreases more or less uniformly over the entire protein range with increasing percentages of immature kernels, but the presence of heavily frosted kernels is related to greater quality decreases in low protein samples than in high protein samples. The correlation between yield of straight flour and loaf volume facilitates simultaneous evaluation of milling and baking quality in grading. The application of these findings to practical grading is discussed.

Introduction

In 1928, owing to limited rainfall following germination, which resulted in uneven growth, and to late heavy frosts, a large portion of the wheat crop contained many types of frost damage together with green and immature kernels. This provided an opportunity for studying the efficacy of the Canadian grain grading system as applied to such wheats and also for determining the relative effects of the various types of damage on milling and baking quality. It was found (5) that the grading system in use that year gave a correct indication of the average relative milling and baking quality of the various statutory and commercial grades, excepting No. 4, which was superior in baking strength to the higher grades.

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The percentages of bran frosted, heavily frosted, immature and green kernels taken individually were not closely related to either flour yield or baking strength, but collectively they were found to exert an appreciable influence. This implied that the total percentage of damage, or conversely the percentage of sound kernels present, would form the most logical basis for the grading of wheat into the commercial grades.

In 1930, amendments were made to the Canada Grain Act introducing changes in the percentage of hard vitreous kernels required in the first four grades and making No. 4 a statutory grade. Frosted wheat may be placed in grades 3 Northern, 4 Northern, No. 5, No. 6 or Feed. In either of the Northern grades it must conform to the statutory definitions* as given in the Canada Grain Act, and in practice it must be comparable with standard samples approved by the Western Committee on Grain Standards as being in accordance with these definitions. The standards for the commercial grades (No. 5, No. 6 and Feed) are set by this committee each year, taking into consideration the general character of the crop. In essence, the grading factors used in the application of these standards to frosted wheat are the weight per measured bushel and the proportion and nature of damaged kernels.

The changes in grading, coupled with the fact that in 1928 the frost occurred when the wheat was nearly mature and extended into areas which do not usually produce frosted wheat, rendered it advisable to conduct similar studies in subsequent years. The present paper deals with the results of surveys of the six crops grown in 1930 to 1935.

Material and Methods

The material for this investigation was supplied by the Western Grain Inspection Division and comprised approximately 15 officially graded samples of each of grades No. 3 Northern to No. 6 in 1930 to 1933 inclusive, and 20 samples of each of these grades in 1934 and 1935. The samples were cleaned,

**Statutory definitions.*

<i>Number and name of grade</i>	<i>Minimum weight per bushel in pounds</i>	<i>Variety of grain</i>	<i>Percentage by weight of hard vitreous kernels, %</i>	<i>Standard of quality</i>
<i>No. 3 Manitoba Northern</i>	<i>57</i>	<i>Red spring wheat of fair milling quality.</i>	<i>25</i>	<i>Reasonably well matured, reasonably free from damaged kernels.</i>
<i>No. 4 Manitoba Northern</i>	<i>57</i>	<i>Red spring wheat</i>	<i>—</i>	<i>Reasonably well matured, but excluded from preceding grades on account of frosted or otherwise damaged kernels.</i>

NOTE: The purity specifications are omitted.

subdivided and forwarded to the cereal research laboratories of the Universities of Alberta and Saskatchewan, and to one laboratory in Winnipeg (1930-1932, University of Manitoba; after 1932, Dominion Grain Research Laboratory).

The following tests were made:

Weight per measured bushel. The procedure used was that previously described (5).

Protein content. Determined by the usual Kjeldahl-Gunning method.

Physical classification. A 50-gram sample was separated by hand-picking into the following classes:

- (a) Sound:—well matured kernels free from frost damage.
- (b) Bran frosted:—kernels showing wrinkling of the bran which did not extend into the crease.
- (c) Heavily frosted:—all kernels showing frost damage not included in (b).
- (d) Immature:—fully formed kernels having a dark color, usually referred to as "bronzy" or "pink" kernels.

In the previous study a separate class was used for "kernels having a decided green color, usually shrivelled." In the present surveys the percentage of such kernels was very small and they were included with the "immature" class.

Some of the samples were found to contain small quantities of broken kernels, foreign matter, etc. This material was discarded and the percentage by weight calculated on the basis of the other groups taken as 100.

There is, of course, a considerable element of judgment in making such a classification and different observers might reasonably arrive at slightly different proportions of the various classes. However, there was frequent rechecking and the results throughout may be regarded as consistent.

Milling test. The samples were experimentally milled in each of the collaborating laboratories, using the technique outlined previously (5), but only a straight flour was produced instead of two grades. In this method standard samples of feed flour and shorts are employed, extraction of the flour from the shorts and the reduction of the feed flour being continued until the residues match the respective standards. The straight grade flour included all the flour with the exception of the feed flour, the two comprising the total. The straight flour does not constitute a constant percentage of the total flour, as the quantity of the former depends partly on the extent to which the feed flour has to be reduced to match the arbitrary standard.

Baking tests. In accordance with the usual policy of the Associate Committee on Grain Research, several baking formulas, designed to reveal the baking characteristics under a range of conditions, were employed. The results of only three of these are reported in detail. The results of two blend tests are used at one point in the statistical examination, but the other four formulas give little additional information. They were used, however,

in the preliminary study of the results. The formulas reported are all based on the "simple" test, which is identical with the A.A.C.C. basic test (3), using mechanical mixing and low-form baking tins.

(a) *Bromate formula*—Simple formula with the addition of 0.001 gm. potassium bromate; employed for all years.

(b) *Malt-phosphate formula*—Simple formula with the addition of 0.3 gm. diastatic malt (approx. 250° Lintner) and 0.1 gm. $\text{NH}_4\text{H}_2\text{PO}_4$; employed for the 1930, 1931 and 1932 crop samples.

(c) *Malt-phosphate-bromate formula*—Combination of formulas (a) and (b); employed for the 1933, 1934 and 1935 crop samples.

(d) *Blend-bromate formula*—Formula (a), but each sample blended with 50% of English flour; employed for 1930, 1931 and 1932 crop samples.

(e) *Blend-malt-phosphate-bromate formula*—Formula (c), but each sample blended with 50% of English flour; employed for 1933 and 1934 crop samples.

Physical Characteristics of the Grades

The mean values for weight per bushel and classes of kernels, arranged according to crop year and grade, together with the standard deviations of single samples, are given in Table I. The standard deviations show that there is a considerable spread in the values which enter into any of the means but that there is no evident trend in the magnitude of this variability with decreasing grade. As the variability affects the reliance that can be placed on the differences between the mean values, the statistical significance of these differences must be estimated before conclusions can be drawn.

When the standard deviation varies from grade to grade the analysis of variance is not strictly applicable since the z distribution will not be realized. Consequently F values (11) are shown only for these cases where the standard deviation is relatively constant. For the others an indication of the significance of the differences can be obtained by comparing them with the standard errors of the means obtained by dividing the standard deviations of single samples given in the table by the square root of the number of samples. Where a definite trend exists, additional reliance may be placed on the reality of the differences.

In general there is a definite lowering of weight per bushel and percentage of sound kernels, and corresponding increases in the percentages of the different forms of damage, from the higher to the lower grades. However, there are exceptions to this general rule. In 1931 grades, No. 5 and No. 6 do not show the expected relation. The differences in weight per bushel and percentage of bran-frosted and immature kernels are not significant. No. 6 has a higher mean value for sound kernels and a lower one for heavily frosted kernels than No. 5, but these differences also may not be significant. These are the only two grades which show a complete departure from the normal relation. Under the system of grading on the basis of weight per

TABLE I
WEIGHT PER BUSHEL AND CLASSES OF KERNELS

Grade†	No. of samples	Weight per bushel		Sound (1)		Bran frost (2)		Heavy frost (3)		Immature (4)	
		Mean, lb.	S.D., lb.	Mean, %	S.D., %	Mean, %	S.D., %	Mean, %	S.D., %	Mean, %	S.D., %
1930											
3°	15	63.8	1.3	63.9	17.1	16.3	16.2	18.3	6.6	1.7	1.0
4°	15	63.7	0.8	43.3	13.8	13.2	6.8	41.9	9.8	1.6	0.6
No. 5	15	62.2	1.5	20.9	10.9	22.2	9.4	51.8	13.0	5.1	3.3
No. 6	14	60.0	1.6	6.9	6.2	33.2	9.5	45.7	17.1	14.3	9.5
	F*	24.13		—		—		—		—	
1931											
3°	15	64.9	1.3	66.4	14.5	7.3	4.2	21.6	11.5	4.7	2.9
4°	15	63.6	1.1	38.2	20.9	10.5	6.2	46.6	19.2	4.8	3.4
No. 5	15	61.6	1.3	19.6	12.8	16.6	12.9	55.1	26.9	8.7	7.1
No. 6	15	61.5	1.8	24.1	14.1	16.0	14.4	50.9	20.2	9.1	7.7
	F*	19.46		24.70		—		7.72		1.92	
1932											
3°	15	64.2	1.4	56.1	12.6	19.4	9.9	23.5	9.5	1.0	0.6
4°	15	63.6	1.2	36.8	12.2	20.3	5.8	40.3	15.6	2.5	2.6
No. 5	15	62.8	1.2	22.2	14.6	19.5	7.2	53.7	20.2	4.6	2.6
No. 6	15	61.5	1.3	7.8	6.3	12.1	6.3	74.7	14.6	5.4	3.8
	F*	11.03		42.75		2.53		27.54		—	
1933											
3°	15	64.2	1.3	70.2	11.4	9.7	5.2	3.9	2.4	4.3	2.3
4°	15	63.5	1.4	49.8	9.8	18.5	10.2	13.6	8.8	7.7	3.8
No. 5	15	63.2	1.5	31.0	13.5	32.2	13.6	19.7	10.3	10.4	5.2
No. 6	15	61.9	1.6	14.2	8.0	29.2	9.5	36.2	17.1	19.6	10.4
	F*	6.78		74.08		18.86		—		—	
1934											
3°	20	64.6	1.2	74.2	6.3	13.0	6.8	1.4	1.0	9.1	6.6
4°	20	63.7	0.9	59.9	9.8	24.3	8.9	4.0	3.2	10.1	4.5
No. 5	20	59.1	1.1	32.9	13.9	32.9	10.7	6.7	7.5	26.0	16.7
No. 6	20	60.8	2.1	12.7	6.9	36.3	14.8	9.5	14.6	41.1	16.4
	F**	28.02		161.52		18.81		—		—	
1935											
3°	20	64.1	2.1	72.5	12.6	14.9	8.4	1.0	0.9	9.2	7.4
4°	20	63.1	2.3	52.0	13.0	25.7	11.4	2.1	1.9	17.4	14.2
No. 5	20	62.2	1.8	32.1	11.6	40.1	17.0	4.6	4.6	22.2	14.0
No. 6	20	59.8	2.1	19.7	9.4	44.8	16.4	5.6	7.0	44.8	18.2
	F**	15.92		77.78		11.27		—		23.66	

* Value of F at 5% point = 2.77.

** Value of F at 5% point = 2.71.

† 3°, 4° = 3 Northern, 4 Northern (Statutory grades).

No. 5, No. 6 (Commercial grades).

bushel and the appearance of the sample, it is inevitable that isolated anomalies in the relative percentages of individual classes of kernels will arise. The grain inspector must balance one form of damage against another. For example, the decreases in the percentage of bran frost from 3 Northern to 4 Northern in 1930, from 4 Northern to No. 6 in 1932, and from No. 5 to No. 6 in 1933, are balanced in each case by increases in the percentage of heavily frosted kernels; the increase in test weight from No. 5 to No. 6 in 1934 is balanced by a marked increase in the percentage of immature kernels; and the decrease in heavily frosted kernels from No. 5 to No. 6 in 1930 is balanced by increases in the percentages of immature and bran frosted kernels and a decrease in the test weight.

On the average the characteristics of the various grades conform closely to those to be expected from an efficient application of the present grading system.

Grade as an Index of Quality

If the grading system is satisfactory the combined milling and baking quality should decrease regularly without overlapping, from the higher to the lower grades. The grade averages for both milling yield and baking value should decrease, but in individual samples high milling yield may compensate for low baking quality, and *vice versa*. Variability in the character of the samples entering any grade would be expected even under an ideal system. Accordingly it is essential that in any discussion of the grading of wheat both milling quality and baking quality should be taken into account.

MILLING QUALITY

Table II shows the mean yields of straight and total flour with their standard deviations and the yield of straight flour expressed as a percentage of the total flour. The standard deviations are reasonably constant for any one year and the analysis of variance can be applied to determine the significance of the differences between the means. The *F* values obtained are in all cases highly significant.

In all years there is a downward trend in yield of straight and total flour as the grade passes from 3 Northern to No. 6. Furthermore, there is a decrease in the percentage of total flour obtained as straight flour. This implies that in commercial practice a greater proportion of the flour from the lower grades of wheat would have to be excluded from the top patents in order to obtain flours approximating in color and ash content those obtained from the higher grades of wheat.

The grade thus gives a reasonably satisfactory indication of the probable milling quality of bulk lots of frosted wheat, though not a clear-cut classification of the individual samples with respect to this character.

BAKING QUALITY

In assessing the baking quality from the results of the baking tests, the loaf volume must be given first consideration, as it gives a direct indication of those qualities desired in Canadian wheat for blending with other wheats

TABLE II
FLOUR YIELD

Grade	No. of samples	Total flour yield		Str. grade flour yield		Str. grade flour as % total
		Mean, %	S.D., %	Mean, %	S.D., %	Mean, %
1930 Crop						
3°	15	72.1	1.7	65.5	0.5	92.3
4°	15	71.7	1.0	64.3	0.9	91.1
No. 5	15	68.5	1.4	62.2	1.8	89.0
No. 6	14	66.0	2.5	60.2	1.1	87.0
	F*	38.9		68.4		
1931 Crop						
3°	15	71.0	1.2	64.7	1.1	91.0
4°	15	70.2	1.2	63.5	1.4	90.4
No. 5	15	67.9	2.5	61.6	2.7	89.9
No. 6	15	67.4	1.9	60.3	2.1	89.4
	F*	12.45		14.29		
1932 Crop						
3°	15	73.0	2.6	66.8	2.9	91.0
4°	15	71.4	1.7	64.9	2.0	90.7
No. 5	15	69.6	1.7	61.9	2.4	88.8
No. 6	15	67.0	2.9	57.8	4.1	86.1
	F*	17.36		24.32		
1933 Crop						
3° ;	15	73.2	0.9	68.8	1.0	94.0
4°	15	72.5	1.5	67.4	1.5	93.3
No. 5	15	71.9	1.8	66.5	1.9	92.8
No. 6	15	69.6	1.4	63.8	1.5	92.0
	F*	17.67		28.02		
1934 Crop						
3°	20	73.2	0.9	69.1	0.8	94.5
4°	20	72.1	1.1	67.6	1.2	93.8
No. 5	20	70.1	2.0	65.0	2.7	92.6
No. 6	20	67.4	1.9	60.9	2.4	90.3
	F†	52.82		8.60		
1935 Crop						
3°	20	73.2	1.9	68.4	1.7	93.5
4°	20	72.0	2.0	66.7	2.0	92.6
No. 5	20	70.5	2.0	64.6	2.2	91.7
No. 6	20	65.6	1.6	58.7	1.8	89.4
	F†	62.45		97.15		

* Value of *F* at 5% point = 2.77.† Value of *F* at 5% point = 2.71.

by millers overseas. With sound wheat a high volume is generally associated with good absorption, and with good texture and appearance of the loaf. In frosted wheat this may not hold and it is necessary to take full account of the other loaf characteristics, since dough quality is often reflected in these.

The mean results of the baking tests with the standard deviations of single samples are given in Table III. There is a great variation of the standard deviations from grade to grade with, in most cases, no apparent trend. Criteria for judging the significance of the differences in Table III can be established in the same manner as for Table I (see page 570). As several characters must be taken into account and as the results are not consistent from year to year, it seems desirable to discuss each year's results separately before arriving at a general conclusion.

1930 Crop

There was practically no difference between grades 3 and 4 Northern. No. 5 was similar to these two grades in volume-producing power, but decidedly below them in general quality, because of the poorer texture and crumb color of the loaves. The absorption was practically the same for grades 3 and 4 Northern, and increased in No. 5 and No. 6. Normally, increased absorption adds to the value of a sample. There is some doubt, however, that increased absorption in the lower grades is as commercially valuable as it is in the higher grades. While greater water-absorbing capacity, regarded as an isolated character, is undoubtedly desirable, in frozen wheat it is generally indicative of undesirable modification of the dough characters and must be taken as an index of the severity of frost damage. We must conclude, therefore, that the average baking quality of the grades was the same for 3 Northern and 4 Northern, slightly inferior in No. 5 and decidedly inferior in No. 6. It follows from this and from the extent of the variability within grades that the grade of the individual samples was not a good index of their baking quality.

1931 Crop

In this year the grade was a very poor index of baking quality of the samples tested. No. 6 had a higher loaf volume than 3 Northern and the loaf characters of the two grades were almost identical. No. 6 had a higher absorption, but this was the only indication that the quality of this grade might have been affected by frost. No. 5 had a higher loaf volume than No. 4, and was slightly better in most loaf characters, with little difference in absorption. Both these grades were inferior to 3 Northern and No. 6. Since the averages of the grades did not fall in the proper order the placing of many of the individual samples for baking quality must have been faulty.

1932 Crop

Grades 3 and 4 Northern were almost identical in baking quality. The results for all the characters placed No. 5 well below 4 Northern, and No. 6 decidedly lower still. Variability increased quite regularly with lowering of

TABLE III
BAKING QUALITY

Grade	No. of samples	Absorption (13.5% M.B.)		General appearance		Crumb color		Crumb texture		Loaf volume	
		Mean, %	S.D., %	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean, cc.	S.D., cc.
1930											
Bromate formula											
3°	15	65.0	0.9	8.5	0.7	7.5	0.2	7.6	0.3	635	45.2
4°	15	65.5	0.8	8.6	0.6	7.3	0.6	7.5	0.5	612	35.7
No. 5	15	67.9	1.3	8.6	1.2	5.8	1.3	6.3	1.6	607	82.6
No. 6	14	71.1	1.2	7.8	1.0	4.7	1.0	5.0	1.2	545	76.4
	F*	94.47		2.61		—		—		4.99	
Malt-phosphate formula											
3°	15	62.8	0.9	9.1	0.5	7.4	0.5	6.9	0.8	658	36.6
4°	15	62.9	1.3	9.0	0.4	6.8	0.8	7.0	0.7	658	29.7
No. 5	15	64.7	1.5	8.6	0.8	4.7	1.3	5.1	1.7	624	79.2
No. 6	14	67.6	1.6	8.0	0.9	3.2	1.1	3.6	1.0	602	74.0
	F*	39.36		7.52		53.73		27.48		2.96	
1931											
Bromate formula											
3°	15	65.2	0.9	8.7	0.8	7.0	0.9	7.2	0.6	640	108.4
4°	15	65.2	1.0	8.5	0.8	6.2	0.7	6.6	0.8	555	61.7
No. 5	15	65.7	0.7	8.6	0.8	6.4	1.1	6.8	1.1	590	86.6
No. 6	15	67.3	0.8	9.0	0.8	6.9	1.0	7.1	0.6	680	93.2
	F*	19.26		0.99		2.41		1.86		5.36	
Malt-phosphate formula											
3°	15	63.1	2.3	8.8	0.7	7.0	0.9	7.3	0.5	500	78.1
4°	15	62.9	2.3	8.3	0.6	6.1	0.7	6.8	0.5	534	52.0
No. 5	15	64.6	1.2	8.3	0.6	6.2	0.9	6.7	0.5	580	60.9
No. 6	15	66.5	1.1	8.7	0.5	6.6	0.6	6.8	0.4	640	66.4
	F*	11.98		2.71		3.63		4.26		3.36	
1932											
Bromate formula											
3°	15	68.1	0.6	9.5	0.3	6.8	0.4	7.3	0.3	739	69.7
4°	15	68.0	0.5	9.2	0.7	6.4	0.7	7.0	0.5	728	82.5
No. 5	15	69.8	1.8	8.5	1.2	5.4	1.0	5.9	1.3	616	109.8
No. 6	15	73.9	1.5	7.8	1.1	4.3	1.1	4.5	1.3	578	148.6
	F*	69.05		—		23.28		—		7.92	
Malt-phosphate formula											
3°	15	69.5	1.2	8.7	0.4	6.5	0.4	6.9	0.3	727	31.8
4°	15	68.9	1.0	9.0	0.5	6.8	0.5	7.1	0.4	748	78.4
No. 5	15	71.8	2.8	8.2	0.9	5.8	0.8	6.0	0.7	624	93.0
No. 6	15	77.4	2.3	7.7	0.8	4.9	1.0	5.7	0.7	571	128.5
	F*	37.68		11.46		10.97		21.57		—	

TABLE III—*Concluded*
BAKING QUALITY—*Concluded*

Grade	No. of samples	Absorption (13.5% M.B.)		General appearance		Crumb color		Crumb texture		Loaf volume	
		Mean, %	S.D., %	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean, cc.	S.D., cc.

1933											
<i>Bromate formula</i>											
3°	15	65.6	1.1	8.9	1.0	5.8	1.2	6.4	1.6	629	81.9
4°	15	65.6	0.9	8.4	1.1	5.2	1.6	5.6	2.1	600	109.6
No. 5	15	66.4	1.2	7.9	1.5	3.8	1.6	4.4	2.0	529	69.4
No. 6	15	68.2	1.3	6.6	1.9	2.4	1.5	3.0	1.7	472	84.8
	<i>F*</i>	15.41		6.66		14.51		9.16		91.40	

<i>Malt-phosphate-bromate formula</i>											
3°	15	62.1	0.8	7.8	0.6	6.0	1.2	6.4	1.2	679	98.3
4°	15	62.3	0.7	7.5	0.9	5.4	1.6	5.6	1.4	648	120.4
No. 5	15	62.9	0.8	7.4	0.9	4.3	1.2	4.7	1.2	563	70.0
No. 6	15	63.7	0.7	6.2	1.3	2.9	1.4	3.2	1.2	488	84.1
	<i>F*</i>	13.62		7.30		14.09		15.93		114.82	

1934											
<i>Bromate formula</i>											
3°	20	64.5	0.8	3.5	1.0	5.3	1.9	5.5	2.1	592	114.5
4°	20	64.6	0.7	3.5	1.1	5.5	2.2	5.5	2.4	601	118.0
No. 5	20	65.4	1.5	3.0	1.7	4.5	2.5	4.5	2.7	515	133.8
No. 6	20	67.1	1.8	2.5	1.7	2.5	2.2	3.0	2.2	444	100.1
	<i>F†</i>	17.51		2.07		7.95		6.54		7.89	

<i>Malt-phosphate-bromate formula</i>											
3°	20	61.7	0.8	3.5	1.0	5.0	2.0	5.0	2.0	714	159.9
4°	20	62.3	0.7	3.5	1.3	5.5	2.2	5.5	2.3	731	179.4
No. 5	20	63.7	1.5	2.5	1.6	4.0	2.2	4.0	2.4	591	173.3
No. 6	20	65.7	1.5	1.5	1.2	1.5	1.2	2.0	1.6	468	115.7
	<i>F†</i>	45.31		8.45		14.81		12.33		11.88	

1935											
<i>Bromate formula</i>											
3°	20	67.6	0.9	4.0	0.3	7.0	0.9	7.5	1.0	693	88.2
4°	20	68.0	0.9	4.0	0.7	6.0	1.3	6.5	1.6	603	104.7
No. 5	20	69.6	1.1	4.0	1.1	5.0	1.8	5.5	1.0	588	98.2
No. 6	20	72.3	1.0	2.0	0.8	2.0	0.9	2.0	0.9	431	61.1
	<i>F†</i>	93.61		36.77		62.60		51.79		29.50	

<i>Malt-phosphate-bromate formula</i>											
3°	20	64.7	0.7	4.0	0.4	6.0	0.9	6.5	0.8	757	101.3
4°	20	65.4	0.7	4.0	0.7	5.0	1.2	5.5	1.5	670	117.4
No. 5	20	67.0	0.9	3.5	1.0	4.5	1.6	4.5	1.7	630	106.3
No. 6	20	69.3	0.7	2.0	0.6	1.5	0.7	1.5	0.8	463	74.6
	<i>F†</i>	152.31		30.18		56.54		123.40		29.88	

* Value of *F* at 5% point = 2.77.† Value of *F* at 5% point = 2.71.

the grade, beginning reasonably low in the Northern grades. The grading failed to differentiate the baking quality of the more lightly frosted grain, but was satisfactory for that more heavily frosted.

1933 Crop

The means show the baking quality to decrease with the grade. The variability in loaf volume was quite high in 4 Northern, and some of the individual samples in this grade undoubtedly had baking quality which should have put them either in 3 Northern or No. 5.

1934 Crop

The results for the average quality were similar to those for 1932. Grades 3 and 4 Northern were almost identical in baking quality. The relatively high variability in all grades for volume, texture and color indicate that the grade was not a good index of the baking quality of individual samples.

1935 Crop

This year was similar to 1933. On the average the grade was a good index of baking quality. The high standard deviations of the first three grades, and particularly of 4 Northern, show that some of the individual samples were wrongly placed.

1930-1935

In two of the six years the grading gave a good indication of the average baking quality. In three years the two higher grades were not properly differentiated but they had better quality than the other two grades, the latter standing in the correct relation to each other. In one year (1931) the grading system failed to give any proper classification with respect to baking quality, and in all years the grade was unreliable as an index of the baking quality of individual samples.

COMBINED MILLING AND BAKING QUALITY

To ascertain the correctness of the grading it is necessary to arrive at a judgment of the combined milling and baking value of the different grades. Obviously if both flour yield and baking quality decrease, or if one of these stays constant and the other decreases, there can be no doubt that the value decreases. Where these two factors vary in the opposite sense, the comparison between the samples will depend on the relative importance attached to yield and to quality in view of the purpose for which the wheat is to be used.

Since Canadian wheat is used under a wide variety of conditions, it is not possible to assign values which will be applicable in all cases. No mathematical relation between the different quality characteristics has ever been established and a definite numerical index of value cannot be calculated. However, by studying the ranking of the grades for milling quality and baking quality separately it is possible to form an opinion of the combined value. To express our opinion in summary form, we have assigned alphabetical values (Table IV) to the grades, using A for 3 Northern and con-

tinuing through the alphabet for progressively decreasing values. The scale was designed so that, if the grading system gave values which decreased with uniform spreads, the placings for grades 3 Northern to No. 6 would be A, D, G, J. The letters intermediate between these are used to indicate intermediate values. Thus, if 4 Northern has a value of B it is considered to be only slightly poorer than 3 Northern.

TABLE IV

APPROXIMATE RELATION OF THE COMBINED MILLING AND BAKING VALUES OF THE GRADES

Grade	Ideal	1930	1931	1932	1933	1934	1935
3°	A	A	A	A	A	A	A
4°	D	B	C	B	C	B	D
No. 5	G	D	C	E	F	F	E
No. 6	J	F	B	J	I	J	J

In every year but 1931 there was a progressive decrease in quality with lowering of grade. In 1933 and 1935 the relation was good throughout; in 1932 and 1934 the spread between 3 and 4 Northern was small, but otherwise the grading was good. In 1930 the spread over the four grades was narrow, but the grades were differentiated nevertheless. It appears that, in most years, the present grading system will classify frosted wheat so that the average quality of the grades will fall in descending order, but not with uniform spreads. In bulk lots the grade is a fairly good index of milling quality but a relatively poor index of baking quality, and with individual samples the indication is less reliable.

Relation between Grading Factors and Quality Characteristics

Any improvement in the grading of frosted wheat must come through more accurate evaluation of the quality of individual samples, since this would bring about a better relation between grades and a reduction in the variability within grades. To find out whether such improvement is possible, it is necessary to ascertain whether the present grading factors are being used to the best advantage and whether there are any additional factors that should be taken into consideration. This can be done by statistical examination of the relation between the various factors and the quality characteristics, over all four grades.*

MILLING QUALITY

In general the simple correlations between percentage of sound or immature kernels or weight per bushel, and straight or total flour yield (Table V) are statistically significant, and while the coefficients are not high enough to permit certain prediction of flour yield, the utility of these factors for grading is apparent. There is little practical difference in the closeness of association

* Readers who prefer to skip the highly statistical argument of the following sections will find the conclusions summarized under the heading "Discussion" on page 589.

for these three factors, and if a single factor were to be used as an index of milling yield the choice would have to be made on the character of the relations as shown by the regression coefficients. However, the multiple correlations between straight and total flour yield, and bran frosted, heavily frosted, and immature kernels show that a more accurate estimate of the yielding capacity of the samples can be obtained if more factors are taken into account. Moreover, since these multiple correlations are higher than any of the simple correlations, it seems preferable to consider the different forms of damage separately rather than to grade on the basis of the total percentage of damage or conversely, on the percentage of sound kernels.

TABLE V
RELATION BETWEEN FLOUR YIELD AND PHYSICAL CHARACTERISTICS OF WHEAT

		Simple correlation coefficients				Simple correlation coefficients	
		Straight flour yield, (s)	Total flour yield, (t)			Straight flour yield, (s)	Total flour yield, (t)
1930 Crop*							
Sound	(1)	.79	.75	Sound	(1)	.46	.41
Bran frost	(2)	-.61	-.71	Bran frost	(2)	-.38	-.49
Heavy frost	(3)	-.35	-.19	Heavy frost	(3)	-.15	-.03
Immature	(4)	-.78	-.84	Immature	(4)	-.50	-.60
Weight per bushel (w)		.78	.84	Weight per bushel (w)		.63	.65
Multiple correlation coefficients				Multiple correlation coefficients			
$R_{s.234} = .86$		5% pt. = .36		$R_{s.234} = .67$		5% pt. = .36	
$R_{t.234} = .92$				$R_{t.234} = .72$			
1932 Crop*							
Sound	(1)	.68	.59	Sound	(1)	.76	.58
Bran frost	(2)	.10	-.03	Bran frost	(2)	-.42	-.32
Heavy frost	(3)	-.56	-.41	Heavy frost	(3)	-.58	-.49
Immature	(4)	-.62	-.72	Immature	(4)	-.70	-.70
Weight per bushel (w)		.60	.57	Weight per bushel (w)		.67	.61
Multiple correlation coefficients				Multiple correlation coefficients			
$R_{s.234} = .81$		5% pt. = .36		$R_{s.234} = .83$		5% pt. = .36	
$R_{t.234} = .82$				$R_{t.234} = .76$			
1934 Crop†							
Sound	(1)	.51	.85	Sound	(1)	.79	.75
Bran frost	(2)	-.28	-.42	Bran frost	(2)	-.12	-.04
Heavy frost	(3)	-.14	-.21	Heavy frost	(3)	-.46	-.35
Immature	(4)	-.47	-.82	Immature	(4)	-.78	-.80
Weight per bushel (w)		.40	.70	Weight per bushel (w)		.81	.86
Multiple correlation coefficients				Multiple correlation coefficients			
$R_{s.234} = .53$		5% pt. = .22		$R_{s.234} = .90$		5% pt. = .22	
$R_{t.234} = .89$				$R_{t.234} = .87$			

* Value of r at 5% point = .26.

† Value of r at 5% point = .22.

The partial regression coefficients describing the relation of flour yield to damage are given in Table VI. As those for bran frost are low and uniformly insignificant, this class of kernels can give no useful indication of yield. The

TABLE VI
RELATION OF STRAIGHT AND TOTAL FLOUR YIELD TO PERCENTAGES OF DAMAGED KERNELS

Year	Partial regression coefficient, % per 1%			Significance, <i>t</i> value (5% point = 1.96)		
<i>Straight flour yield</i>						
	b_s 2-34	b_s 3-24	b_s 4-23	b_s 2-34	b_s 3-24	b_s 4-23
1930	-.07	-.09	-.40	0.99	2.22	3.14
1931	-.04	-.05	-.27	1.05	2.49	3.12
1932	-.07	-.11	-.70	0.68	2.86	2.79
1933	-.03	-.06	-.16	0.90	2.31	2.93
1934	-.08	-.10	-.16	1.32	1.07	3.41
1935	-.07	-.19	-.18	1.54	1.25	5.11
<i>Total flour yield</i>						
	b_t 2-34	b_t 3-24	b_t 4-23	b_t 2-34	b_t 3-24	b_t 4-23
1930	-.06	-.10	-.32	1.15	2.94	3.13
1931	-.06	-.03	-.24	1.48	1.92	3.13
1932	-.06	-.06	-.66	0.90	2.21	3.66
1933	-.01	-.04	-.14	0.34	1.92	3.30
1934	-.05	-.07	-.12	1.56	1.38	5.08
1935	-.04	-.16	-.15	1.12	1.26	5.16

s = straight flour yield. *t* = total flour yield. 2 = bran frost. 3 = heavy frost. 4 = immature.

relation of heavy frost is more definite, particularly with straight flour yield, and the coefficients for immature kernels are significant throughout. The differences between the regression coefficients in a single year are insignificant in two-thirds of the comparisons, but on the average of the six years they are significant. The greatest decrease in flour yield for a unit increase in damage is obtained with immature kernels, followed by heavy frost, while the effect of bran frost is negligible.

The weight per measured bushel is related to the percentage of damage in the samples (Table VII). The association is closest with immature kernels and, with the exception of 1930, negligible or statistically insignificant with bran frost. The regression coefficients show too that the effect of the different forms of damage on weight per bushel is very similar in general character to their effect on flour yield. This similarity is particularly valuable as it relates two separately determined indices of flour yield.

It has been pointed out that the present grading gives a fair indication of flour yield, and the utility of the weight per bushel can be judged from a covariance analysis of weight per bushel and flour yield (Table VIII). The

TABLE VII
RELATION OF WEIGHT PER BUSHEL TO PERCENTAGE OF DAMAGED KERNELS

Year	Bran frost (2)	Heavy frost (3)	Immature (4)	Significance		
Simple correlation coefficients				Value of <i>r</i> at 5% point		
	<i>r_{w2}</i>	<i>r_{w3}</i>	<i>r_{w4}</i>			
1930	-.64	-.19	-.84	.26		
1931	-.28	-.37	-.49	.26		
1932	-.05	-.53	-.44	.26		
1933	-.23	-.60	-.36	.26		
1934	-.26	-.41	-.62	.22		
1935	-.05	-.36	-.74	.22		
Partial regression coefficient, lb. per 1%				<i>t</i> . value (5% point = 1.96)		
	<i>b_{w2-34}</i>	<i>b_{w3-24}</i>	<i>b_{w4-23}</i>	<i>b_{w2-34}</i>	<i>b_{w3-24}</i>	<i>b_{w4-23}</i>
1930	-.03	-.03	-.21	0.99	1.32	3.38
1931	-.01	-.06	-.27	0.29	3.75	3.75
1932	-.07	-.05	-.16	1.97	3.53	1.84
1933	-.01	-.06	-.04	0.35	3.30	1.69
1934	-.01	-.10	-.07	0.47	2.99	4.29
1935	-.01	-.16	-.10	0.29	1.74	4.65

TABLE VIII
RELATION OF WEIGHT PER BUSHEL AND FLOUR YIELD WITHIN AND BETWEEN GRADES

Year	Simple correlation coefficients								
	Straight flour yield (s)			Total flour yield (t)			5% point		
	<i>r_{ws}</i>			<i>r_{wt}</i>					
	Total all grades	Between grades	Within grades	Total all grades	Between grades	Within grades	Total all grades	Between grades	Within grades
1930	.78	.97	.43	.84	.98	.63	.26	.95	.26
1931	.63	.99	.33	.65	.97	.39	.26	.95	.26
1932	.60	1.00	.27	.57	1.00	.26	.26	.95	.26
1933	.67	1.00	.50	.61	.99	.42	.26	.95	.26
1934	.40	.99	.07	.70	.99	.28	.22	.95	.22
1935	.81	1.00	.71	.86	1.00	.80	.22	.95	.22

significant correlations between grades show satisfactory differentiation, but the significance of most of the "within grades" correlations indicates that it is possible to reduce the variability through stricter limits for test weight.

Milling quality cannot be judged solely by flour yield. The ease with which the flour can be separated from the bran is also an important aspect of quality. Some measure of this can be obtained from the relative yields of "total" and straight flour. If the simple correlations (Table V) between the two types of flour and the percentage of heavily frosted and immature

kernels are compared, we find that, on the whole, immaturity is more closely associated with total flour yield than with straight flour yield, while the reverse is true for the percentage of heavily frosted kernels. It is probable that immaturity is related primarily to the proportion of bran while the percentage of heavily frosted kernels is more closely related to the ease of separation.

BAKING QUALITY

Although loaf volume is not the sole measure of the complicated and inter-related group of characteristics known collectively as baking quality, it is a good index of "strength" which is so desirable in Canadian wheat. Therefore, the utility of grading factors can be best studied by examining their relation to loaf volume.

The simple correlations (Table IX) of the various classes of kernels with loaf volume are all low and in the first two years of the survey many of them are statistically insignificant. Even the best relation, that with the percentage of sound kernels, is too low to provide a reliable basis for grading with respect to baking quality. The multiple correlations showing the combined relation of bran and heavy frost and immature kernels to loaf volume are slightly higher than the correlations obtained with the sound kernels. Thus grading taking into account the individual kinds of damage might be slightly better

TABLE IX
RELATION BETWEEN LOAF VOLUME AND CLASSES OF KERNELS

Year	Simple correlation coefficients					Multiple correlation coefficients	
	Sound (1)	Bran frost (2)	Heavy frost (3)	Immature (4)	5% point	$R_{.234}$	5% point
<i>Bromate formula</i>							
1930	.40	-.36	-.11	-.47	.26	.51	.36
1931	.17	.14	-.30	.28	.26	.35	.36
1932	.63	.32	-.64	-.35	.26	.70	.36
1933	.47	-.49	-.48	-.31	.26	—	—
1934	.53	-.38	-.26	-.40	.22	—	—
1935	.60	-.31	-.32	-.42	.22	—	—
<i>Malt phosphate formula</i>							
1930	.36	-.40	-.03	-.43	.26	—	—
1931	.06	.12	-.19	.26	.26	—	—
1932	.60	.24	-.58	-.36	.26	—	—
<i>Malt phosphate bromate formula</i>							
1933	.53	-.50	-.49	-.41	.26	.67	.36
1934	.58	-.40	-.28	-.44	.22	.60	.31
1935	.61	-.36	-.32	-.41	.22	.65	.31

than grading on the basis of the percentage of sound kernels alone, provided the partial regressions of loaf volume on percentage of kernels were known in advance for each class of kernel, a condition not realizable in practice. In any event, the comparatively low values of the multiple correlations indicate that other factors affecting loaf volume have been left out of consideration.

It was shown (5) that the percentage of protein had an important effect on the baking quality of frosted wheat of the 1928 crop. It is well established that the major factor affecting the capacity of sound Canadian hard red spring wheat to produce loaf volume is the protein content. Immaturity and frost, by deteriorating protein quality, may diminish this relation but need not destroy it.

The soundness of this view is demonstrated by the results of an analysis of the covariance of loaf volume and protein content (Table X). The simple correlations for all grades are, on the whole, higher than the multiple cor-

TABLE X
RELATION OF WHEAT PROTEIN AND LOAF VOLUME WITHIN AND BETWEEN GRADES

Year	Simple correlation coefficients			5% point		
	Total all grades	Between grades	Within grades	Total all grades	Between grades	Within grades
<i>Bromate formula r_{6b}</i>						
1930	.75	.57	.80	.26	.95	.26
1931	.83	.90	.80	.26	.95	.26
1932	.83	.83	.97	.26	.95	.26
1933	.78	.96	.76	.26	.95	.26
1934	.89	.89	.91	.22	.95	.22
1935	.62	.74	.74	.22	.95	.22
<i>Malt-phosphate formula r_{6c}</i>						
1930	.65	.20	.67	.26	.95	.26
1931	.68	.94	.58	.26	.95	.26
1932	.69	.44	.63	.26	.95	.26
<i>Malt-phosphate-bromate formula r_{6d}</i>						
1933	.81	.97	.80	.26	.95	.26
1934	.88	.89	.92	.22	.95	.22
1935	.62	.68	.75	.22	.95	.22

relations involving the three classes of damage (Table IX) or, in other words, the protein content gives a better indication of baking quality than the factors now used in grading. However, the relation is by no means perfect. The fact that the "between grades" simple correlation coefficients are not significant, and that the "within grades" correlations tend to be higher than the correlations over all grades, indicates that the relation between protein and loaf volume is affected by the proportion of damage in the sample, and that the relation can be improved if this is taken into account.

TABLE XI

RELATION BETWEEN LOAF VOLUME, AND FORMS OF DAMAGE AND PROTEIN (MULTIPLE CORRELATION)

Year	Multiple correlation coefficients		
	Bromate formula $R_b .2346$	Malt-phosphate-bromate formula $R_d .2346$	5% point
1930	.90	—	.40
1931	.85	—	.40
1932	.92	—	.40
1933	.86	.90	.40
1934	.99	.99	.33
1935	.77	.83	.33

2 = bran frost. 3 = heavy frost. 4 = immature.
6 = wheat protein.

TABLE XII

RELATION BETWEEN LOAF VOLUME, AND DAMAGED KERNELS AND PROTEIN CONTENT (PARTIAL REGRESSIONS)

Year	Partial regression coefficient, cc. per 1%			
	$b_v 2.346$	$b_v 3.246$	$b_v 4.236$	$b_v 6.234$

Bromate formula

1930	-0.6	0.1	-4.1	60.6
1931	-3.9	-0.6	14.2	93.6
1932	0.4	-1.8	-3.4	63.3
1933	-1.2	-2.4	-0.7	52.8
1934	-1.9	-12.7	1.3	70.7
1935	-3.2	-1.2	-3.4	52.8

Malt-phosphate formula

1930	-1.0	0.2	-2.7	47.0
1931	-1.5	-0.3	7.1	37.2
1932	-0.4	-1.8	-5.0	43.5

Malt-phosphate-bromate formula

1933	-1.0	-2.3	-2.3	61.8
1934	-3.7	-6.6	-1.7	85.1
1935	-4.0	-0.8	-3.8	58.7

v = loaf volume. 2 = bran frost. 3 = heavy frost. 4 = immature. 6 = protein content.

The multiple correlation coefficients showing the combined relation of protein and the different classes of damage with loaf volume (Table XI) are higher than any of the other correlations studied, and they show that from 60% to 98% of the variance in loaf volume, depending on the year, can be predicted from a knowledge of the protein content and the percentages of bran frosted, heavily frosted and immature kernels in the sample. This would be sufficiently close for grading purposes, but this level of accuracy can only be attained if the relation of these factors to loaf volume is known in advance. The great variability in the partial regressions (Table XII) makes it impossible to arrive at even a reasonable estimate of the relative effect of these factors on the basis of the experience of past years, and an actual determination of the partial regressions which could not be made before the crop year is well advanced would only be of academic interest.

The variation in the partial regressions of loaf volume on protein and the known differences in the general character of the damage in different years (Table I) made it seem possible that the relation is a complex one worthy of further study. Accordingly, the simple regression coefficients of loaf volume on

protein content were calculated for each grade and year and are given in Table XIII, and graphically, for the bromate formula only, in Fig. 1. In 1930 and 1932 the differences between the regression coefficients are highly significant. For the bromate formula in 1931, for the malt-phosphate-bromate formula in 1934 and for both formulas in 1935, they are just above the 5% level of significance and the remainder of the differences are not significant. In addition, there are some marked differences between the regression coefficients for the same grade and formula in different years. The graphs show that the regression lines differ not only in slope but also in general level.

TABLE XIII
RELATION OF PROTEIN CONTENT OF WHEAT AND LOAF VOLUME BY GRADES

Year	Simple regression coefficient, cc. per 1%				Significance of differences between regression coefficients	
	3°	4°	No. 5	No. 6	F	5% point
<i>Bromate formula</i>						
1930	40.9	38.6	83.8	85.6	5.27	2.79
1931	74.9	36.2	64.0	97.6	3.09	2.79
1932	42.6	61.0	61.9	124.0	6.35	2.79
1933	52.1	66.4	39.3	57.2	0.80	2.79
1934	63.7	56.3	64.8	50.7	1.04	2.74
1935	47.4	57.8	67.6	21.0	2.81	2.74
<i>Malt-phosphate formula</i>						
1930	28.5	17.2	66.9	76.0	5.13	2.79
1931	40.0	7.1	33.1	55.0	1.28	2.79
1932	3.8	40.3	47.9	120.8	16.61	2.79
<i>Malt-phosphate-bromate formula</i>						
1933	62.8	74.0	45.2	64.8	0.78	2.79
1934	89.2	85.2	87.2	58.0	3.26	2.74
1935	55.2	63.9	76.7	23.8	2.77	2.74
<i>Blend bromate formula</i>						
1930	20.1	22.3	39.8	45.8	2.20	2.79
1931	35.0	4.8	25.3	49.2	3.14	2.79
1932	35.0	31.0	30.1	63.5	2.80	2.79
<i>Blend malt-phosphate-bromate formula</i>						
1933	25.2	29.7	19.0	35.6	0.99	2.79
1934	35.0	35.4	39.2	25.8	2.53	2.74

The differences in slope might be the result of differences in the damage distribution over the protein range for different grades. This was checked by calculating the simple correlation and regression coefficients of the percentage of heavily frosted and immature kernels on protein content. In 1931, No. 5 gave a correlation of -0.45 with a regression of -10.4 , indicating that the line shown for this grade on the graph slopes more steeply than it would if the protein-damage distribution were uniform. The other lines are unaffected.

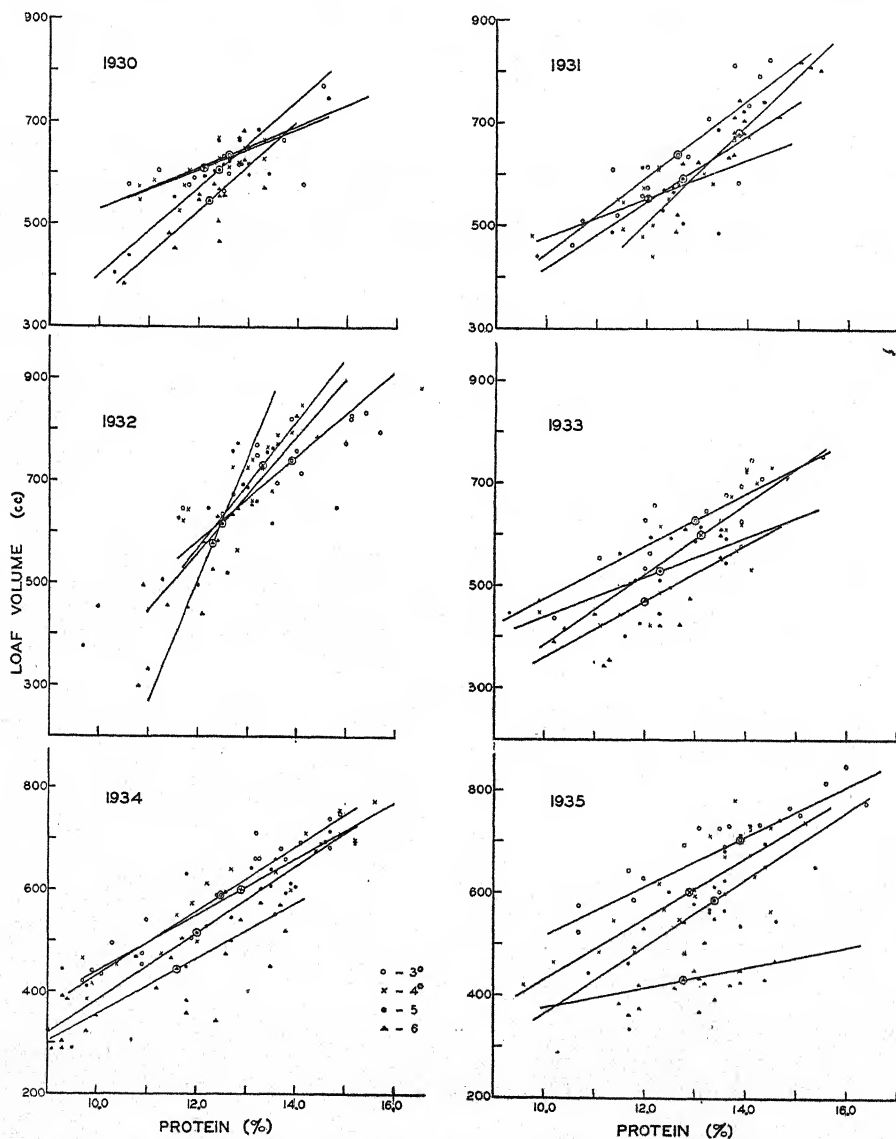


FIG. 1. Simple regression of loaf volume and protein content (bromate formula).

Provided that gas production is not a limiting factor, the differences in slope and level of the lines of regression of loaf volume on protein quantity must be related to differences in the protein quality in the widest sense of this term, including not only the character of the protein as originally present in the flour, but also the factors which may modify this character during fermentation. The bromate formula does not guarantee adequate gas production, but the similarity in character of the regression diagrams obtained with the malt-phosphate and malt-phosphate-bromate formulas and those for the bromate formula indicates that gas production was not a limiting factor. The relation between the slope and level of a regression line and protein quality is complex and direct interpretation is not easy. If two regression lines have different general levels the lower one must represent a lower protein quality, as equal quantities of protein do not produce equal loaf volumes. If lines have different slopes it indicates that the relative protein quality changes with the amount of protein present, or that the quality represented by the steeper line is so poor that gas retention is deficient in the lower protein range. One might hazard a guess that where the general levels differ the characteristics of the protein as originally present in the flour are different over the entire protein range and, in the second case, that the deleterious factors operative in the dough are different in kind or degree, and when the protein content is sufficiently high the net deleterious effect is not as serious as when the protein content is so low that good colloidal character would be necessary to maintain normal expansion. We have no experimental evidence to support this hypothesis, but the fact that none of the regression lines have a negative slope is not inconsistent with it. In the actual regression diagrams, of course, both slope and level may differ simultaneously.

In an attempt to relate the character of the regression lines to the physical character of the samples the multiple regression of these regression coefficients on the average percentage of bran frost, heavy frost and immature kernels for each grade was calculated but was found to be insignificant. However, the coefficient for heavy frost was considerably higher than for the other two classes of kernels and the simple correlation between this kind of damage and the regression of loaf volume on protein content for each grade was found to be 0.53, with a regression coefficient of 0.51, both of which are statistically significant. Thus we can conclude that the slope of the line representing the relation between protein and loaf volume in each grade becomes steeper as the average amount of heavy frost damage increases, and that the effect of bran frost and immature kernels is negligible. In other words, heavily frosted kernels have an effect on "gluten quality" which is markedly deleterious to loaf volume when the protein content is low, and less evident, or even absent, when the protein content is high.

Since a large proportion of Canadian wheat is used for blending it is of interest to ascertain the effect on this relation of the reduction of protein content by blending. The simple correlation and regression coefficients describing the relation between the coefficients of the regression of loaf volume

on protein content derived from the results of the bromate and malt-phosphate-bromate tests, and those derived from the corresponding blend tests, were found to be $r = 0.80$, $b = 0.40$, both of which are highly significant. The variation with protein content in the effect of heavily frosted kernels can be seen in blended flour, but its magnitude is reduced roughly in proportion to the amount of weaker flour used in the blend.

In the last three years of the survey the differences between the slopes of the regression lines are insignificant, or just barely significant, while there are pronounced differences in their general level, and thus we were able to study the relation of the different forms of damage to the level of the loaf-volume-protein regression lines, relatively freed from the complication of differences in slope. The level of each regression line was taken as the loaf volume at the point of intersection of the regression line with the ordinate for the average protein content of all samples in that year. Grade 3 Northern was taken as standard and the levels expressed as differences between this grade and No. 4 Northern, No. 5 and No. 6, for each year, and correlated with the corresponding differences in the percentage of immature kernels, giving a correlation coefficient of $+0.83$, which is highly significant. It appears that immaturity has a detrimental effect on "gluten quality" which is independent of the amount of protein.

Thus there are three factors which can be used simultaneously as an indication of the loaf volume—protein, percentage of immature kernels and percentage of heavily frosted kernels. Since it has been shown that immaturity and heavy frost are both related to gluten quality these factors should also give an indication of the wider character, baking quality, including dough characters and the shape and texture of the loaf. This receives support from the general relation which can be seen in Table I and Table III, and from the significant simple correlation (0.59) between the sum of the percentages of heavily frosted and immature kernels and the texture score which was calculated, 3 Northern being used as a standard in a similar manner to that given in the last paragraph. The crumb color score is also affected by these forms of damage.

TABLE XIV

RELATION BETWEEN YIELD OF
STRAIGHT FLOUR AND LOAF
VOLUME (BROMATE FORMULA)

Year	Simple correlation coefficient	5% point
1930	.58	.25
1931	.08	.25
1932	.70	.25
1933	.48	.25
1934	.67	.22
1935	.63	.22

COMBINED MILLING AND BAKING QUALITY

The grade ought to give an indication of both milling quality and baking quality and it is important to ascertain whether specifications ensuring a good indication of the one can be consistent with good indication of the other. Milling quality is related to weight per bushel, and to the percentages of heavily frosted and immature kernels. Baking quality is related to protein content and the percentages of heavily frosted and immature kernels. Thus there are two common factors and a third (test weight) which is related to these two. There should,

therefore, be a fair degree of correspondence between the variation in the two qualities. The simple correlations between yield of straight flour and loaf volume (Table XIV) are highly significant, with the exception of that for 1931, and this should facilitate the simultaneous indication of milling and baking quality by a single grade designation.

Discussion

The system of grading frosted wheat on the basis of the weight per measured bushel and the nature and extent of the damage to the appearance of the kernels is applied by the inspectors of the Western Grain Inspection Division in a satisfactory manner. Any major weaknesses in the grading of this class of Canadian wheat cannot be attributed to deficiencies in the application of the system.

The average milling quality of frosted wheat decreases with grade in every year, but there are marked discrepancies in the baking quality. However, with the exception of 1931 the average combined milling and baking value decreases as the grade lowers, though the spreads between the grades are by no means uniform. If the system were perfect, both milling quality and baking quality should decrease regularly from grade to grade when the average of the crop is considered. This ideal is far from being realized. There is great variation in the quality of the individual samples entering into any grade, and this variation is more pronounced in the baking quality than in the milling quality, and might even be described as excessive. In practice this situation means that the importer purchasing large bulk lots of frosted wheat has much more assurance that the grade will be a true indication of the combined milling and baking quality than has the farmer delivering a single wagon or carload, because the former quantity approaches more nearly the average of the grade for any crop year. The grading of individual frosted samples stands in great need of improvement, particularly in bringing the grade in harmony with the baking quality. If this can be done the regularity in quality of export would also be improved.

The avenues by which improvement might be sought are strictly limited by practical considerations. Speed is a necessity in the grading of Canadian wheat. On October 1, 1928, the Western Grain Inspection Division graded 3,787 cars of grain in a single day, and the inspections of wheat at Winnipeg alone commonly number 1,700 per day during the rush season. Simplicity of method and equipment and reasonable cost of the latter are highly desirable, as unofficial grading by the operators is necessary for a substantial portion of the grain handled by nearly 6,000 country elevators. No method of testing wheat which cannot meet these requirements is worthy of consideration as a basis for grading. This rules out the Brabender Farinograph or methods similar to the Pelshenke test, and in addition these methods have been shown to be unsatisfactory, even in the hands of skilled technicians, when used with Canadian hard red spring wheat (1, 4). Furthermore, any radical change in the basis of grading would lead to a period of confusion in

the trade until the precise effect of the change was thoroughly understood by all interests. These considerations led us to the view that the factors now in use should be utilized so far as possible.

The most satisfactory indications of the milling quality of frosted wheat can be obtained either from the weight per measured bushel or from the percentages of heavily frosted and immature kernels. The latter class of damage is somewhat more deleterious than the former. The test weight is influenced in a similar manner. Some improvement in the indication of milling quality might be effected through the adoption of stricter limits for test weight. To estimate the baking quality three factors must be used: protein content, and the percentages of heavily frosted and immature kernels. The use of damage alone as at present is not satisfactory; on the other hand, while protein is the best single index of baking quality, the relation is improved if damage is taken into account. Immaturity is equally undesirable over the entire protein range, but the bad effect of heavy frost in a sample is comparatively small if the protein content is high, and very pronounced if the protein content is low; this relation persists even when the protein content is reduced by blending. It is probable, in the light of the work of Newton and McCalla (9), that the protein content gives a rough indication of what the baking quality would have been if the sample had been mature and unfrozen; while the physical appearance of the kernels is related to the decrease from this quality level due to frost injury. The presence of immature kernels in a sample has a double significance since immaturity is deleterious, even without frost, and the harmful effect of frost increases with the immaturity of the wheat at the time of freezing (10). The percentage of immature kernels increases, in a general way, with both immaturity at freezing and the severity of the frost (8).

It will be noted that two of the factors are related to both milling quality and baking quality. This is most fortunate as it greatly facilitates the simultaneous evaluation of these two characteristics which differ so greatly in their nature.

The complete application of these findings in practice is dependent on the protein content being made a factor in the grading of wheat. The introduction of a protein requirement in the grade specifications, or even its indication as a notation on the certificate is fraught with very great difficulties (6, 7) and it does not seem that the advantages to be gained would compensate for the expense and complication of trade practice involved. However, there is another possible method by which the protein content could be taken into account. The wheat-growing region of Western Canada can be divided into zones: the open prairie, and the area where gray-wooded soils predominate, lying mostly in the northern section of the Western Provinces, with a transition zone between these two. The annual protein surveys of the Laboratory of the Board of Grain Commissioners (2) have established that the protein content of wheat in the first zone is generally high and that of the second definitely lower. The separation of the two main zones for grading purposes

would greatly facilitate the evaluation of quality from the appearance of the samples. This proposal will require careful and detailed study, but the use of a zoning system in Argentina is evidence that it is not outside the range of practicability. It is not feasible, of course, to apply a zoning system for the grading of frosted wheat alone, and its application to this class of wheat would have to be considered as part of a general revision of the wheat-grading system. A discussion of this wider problem is beyond the scope of this paper.

Of the factors now in use the greatest importance should be attached to the percentage of immature kernels, with somewhat lesser emphasis on the percentage of heavily frosted kernels. The percentage of bran frost can be disregarded because if this form of damage is present alone it indicates that the frost to which the grain was exposed was not severe and that it occurred late in the development of the plant and, consequently, there was no real injury to either milling or baking quality (10); if, on the other hand, a sample contains, in addition, heavily frosted and immature kernels, these forms of damage are the only ones that need be considered. The weight per measured bushel, which is always determined in grading, can be used to supplement the estimation of damage. While refinement in the application of the present grading factors might effect a minor advance, no major improvement can be expected unless the protein content is taken into account.

The results of the six years' survey are essentially in agreement with those of the study of the 1928 crop (5). The importance of the protein content as a measure of baking quality and the utility of weight per measured bushel as an index of milling yield are common to the conclusions from both investigations. In the present study the significance of the percentages of heavily frosted and immature kernels was elucidated through the use of methods of statistical analysis inapplicable to the results of a single year, and our earlier conclusion that the proportion of sound kernels was just as informative as as the percentages of the individual forms of damage has, therefore, been revised.

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